MATERNAL EFFECTS AND COLD ADAPTATION IN MICE

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STATEMENT

Except where acknowledgements are made in the text, all the material contained in this thesis is the work of the candidate.

R. G. Rice

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1.SUMMARY

The involvement of maternal effects in the processes of adaptation to cold by mice was examined experimentally.
Methods:

1. Wild mice were trapped and bred in a laboratory at room temperature. From this original population two groups were derived and bred concurrently for 12 generations, one group at 23° C (the controls) and the other at 3° C (the Eskimos). Body measurements, organ weights and reproductive performance were recorded in each generation. The two populations were also compared, in the same environment, (a) by transferring controls to the cold at generations 5 and 9 and by (b) transferring Eskimos to the warm at generation 5.

2. Maternal effects were analysed in two separate experiments.

(a) At generation 6, in both temperatures, young of transfers and indigenes were cross-fostered at birth. The effects of the different postnatal environments on the growth and later reproductive performance of the fostered mice were assessed.

(b) At generation 1Ø in the cold, transfers and indigenes were reciprocally mated. The two classes of hybrid young so produced were assumed to be genetically identical. The effect of the different pre- and postnatal maternal environments on the growth and later reproductive performance of the hybrids was analysed.

3. The quality and quantity of the milk of Eskimos and controls in the cold, and controls in the warm, were recorded at generation 1Ø.

Findings:

1. Eskimo mice gradually became heavier, longer and fatter than the controls over the generations. Eskimo kidneys were heavier than those of the controls from the first generation onwards. Eskimo tails were always

shorter than controls but they became longer over the generations, but ratio of tail to body length remained constant. Adrenal weights did not differ significantly between the two groups in any given generation. There was a rapid decrease in adrenal weights of Eskimos and their variability at generation 5. Reproductive performance of the Eskimos was initially inferior to that of the controls, but there was gradual improvement, due in part to a gradual decrease in the delay between mating and the onset of breeding. By generation 7 there was no difference between the two groups in any measure except for infant mortality which was always higher among the Eskimos. Growth of Eskimo young in the early generations was slower than controls but gradually became faster over the generations. By generation 1Ø Eskimo young grew much faster than controls and were also fatter. Transfers of controls to the cold at generations 5 and 9 repeated the initial transfer in most respects, but the effects of cold were not as severe. The Eskimos transferred to the warm at generation 5 showed no superiority over controls in reproductive performance but their growth was slightly superior.

However, in the first few generations Eskimo adrenal weights were highly variable and were heavier than the controls when the first few generations were pooled.

2. Maternal effects were evident in the experiments, both on cross-fostering and on hybridizing.

(a) There were only slight differences due to foster-parents in the warm. In the cold, where the differences between parents were greater, there was a strong depressing effect of control foster-parentage on the growth of Eskimo mice; and this was still evident in the growth of the offspring of these mice. There was no growth-enhancing effect of Eskimo foster parentage on control young. The later reproductive performance, of the latter was, however, affected. The litter size of the controls with Eskimo foster-parents was greater than those with control foster-parents.

(b) There was a strong effect of combined pre-and postnatal environment on the hybrid mice. Hybrids with

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Eskimo mothers grew faster and had a reproductive performance superior to that of those with control mothers. In fact differences in growth up to 21 days were determined entirely by the type of mother. There was, however, only a slight, if any, effect of Eskimo mothering on the subsequent generation.

3. Milk supply was strongly correlated with pup weight. There was an ontogenetic effect of temperature on supply: pups of mice in the cold, whether Eskimo or control, drank half as much again as controls in the warm. There was a phylogenetic effect on milk quality: Eskimos had richer milk than controls in either temperature.

Discussion:

The value of the present study as an example of micro-evolution is discussed. One of the major deficiencies of the experiment was the lack of replicate populations.

Increase in body weight in the cold was one of the main adaptive changes in the cold. In nature many other factors in the environment act to modify changes in weight.

Large adult body size was largely due to rapid pre-weaning growth of the Eskimo mice.

Maternal effects were evident in the early growth of the young but the situation was complex, involving effects of the young on the mother. The capacity of the mother is perhaps the limiting factor in early growth.

Further analyses of maternal effects are proposed including the use of standardized, artificial mothers, egg transfers and cross-fostering at successive generations during the process of adaptation.

2. INTRODUCTION

2.1 Outline of Present Study

The most characteristic feature of mammals is the close physical association between the young and their mother. Not only is there considerable growth and development of the young in utero, but for a time postnatal growth depends entirely on the milk provided by the mother. So it is often difficult to distinguish between the effects of genetical variation among the young and the effects of the pre- and postnatal environment provided by the mother, that is, maternal effects. It is possible, at least in theory, for maternal effects to act over successive generations resulting in a cumulative effect which can simulate genetical change (Fig. 2.1).

Consider that P1 is the first generation in a new environment. The environment affects P1 to produce an altered phenotype. The F1 generation is also influenced by the environment and (via maternal effects) by the altered phenotype of P1. Similarly F2 is also affected by the environment and by F1 (maternal effects) and indirectly by the influences (environment and maternal effects) affecting F1. The thickening arrows indicate how these maternal effects might accumulate over several generations.

By this mechanism advantageous characteristics aquired during an animal's lifetime could be passed on to succeeding generations. My hypothesis is that maternal effects are significant in the adaptation of mammals to a new environment.

The house mouse colonises a wide range of environments. It is highly adaptable and, although it is better known as a commensal species, independent populations are quite common in many seemingly adverse environments (Berry & Jakobson, 1975). For a mammal of

its size cold is one critical factor. When colonizing a cold environment considerable adaptation to that environment would be expected.

The general aim of the present project is to analyse maternal effects in a house mouse population recently adapted to a cold environment. More specific aims are as follows:

1. To examine some of the overt structural and physiological changes that occur during the long-term adaptation of a house mouse population to cold.

2. To distinguish between maternal and genetical effects and their contribution to differences between warm-adapted and cold-adapted populations.

3. To examine aspects of the postnatal maternal environment of cold-adapted and warm-adapted mice.

The experimental design is summarized in Fig. 2.2. Wild mice were trapped and brought into the laboratory to found a breeding colony at 21°C. This was generation Ø. Offspring of the trapped mice were mated and half were placed in a cold room at 3°C;the other half remained at 21 C. Thus the founders of the cold-adapted and warm-adapted populations were from the same original stock. The two populations were then bred concurrently for 11 generations.

To estimate the extent to which the two populations had diverged from each other genetically, they were compared in the same environment. Some mice from the cold-adapted population were transferred to the warm at mating. The offspring of these mice were then compared with the warm adapted mice. Similarly some mice from the warm adapted population were transferred to 3°C and their offspring compared with the cold-adapted mice. Transfers were made at generations 5 and 9.

Differences between the warm-adapted and cold-adapted mice observed in the same temperature are

assumed to be due to
(i) genetical change (from selection by cold and drift)
or (ii) cumulative maternal effects.

One way to distinguish the contributions of maternal and genetical components is to cross-foster. Cross-fostering at birth makes possible separation of postnatal maternal effects from the combined contribution of prenatal maternal effects and genetical differences. At generation 6, cross-fostering was therefore carried out between the cold-adapted and warm-adapted populations in both temperatures.

Combined pre- and postnatal maternal effects can be assessed by reciprocal mating. Cold-adapted and warm-adapted mice were mated reciprocally to produce two classes of hybrid: one with cold-adapted mothers and one with warm-adapted mothers. The assumption is that these two classes are genetically identical and that differences between them can therefore be attributed to their contrasted maternal environments.

Mice of generation 10 were reciprocally mated.

It is one thing to demonstrate the existence of maternal effects, but it is more difficult to identify the effects and how they operate. To begin to do this it is necessary to examine aspects of the maternal environment.

Variations in prenatal maternal environment which affect growth and development of young include changes in uterine blood supply and in size and elasticity of the uterus (Maclaren & Michie, 1960). Aspects of the postnatal maternal environment which affect the growth and development of the young are: (i) maternal activity, including (a)behaviour directed towards the pups and (b)other behaviour such as nest building; (ii)the quantity and composition of the milk. Experiments on milk supply were carried out with generation 10.

The experimental design outlined above allows for

quantitative analysis of maternal effects involved in the adaptation of house mice to a cold environment. The demonstration of substantial maternal effects should provide evidence for the thesis that these effects are significant in the adaptation of mammals to a new environment.

2.2 Background

I give below a brief coverage of some of the literature related to cold adaptation, maternal effects and natural mouse populations. Relevant papers are also referred to throughout the text.

2.2.1 Cold adaptation of mice.

Patterns of cold adaptation have been extensively investigated in mammals (reviewed by Heroux (1961), Hart (1971), Barnett and Mount (1967) and Chaffee and Roberts (1971). Particular attention has been paid to 'non-shivering thermogenesis' (Jansky, 1973).

Adaptation to the environment occurs at two levels: ontogenetic adaptation - adaptation of the individual during its lifetime; and phylogenetic adaptation - adaptation of a population or species over many generations.

The ontogenetic response of a mouse to cold varies according to the length of exposure (Barnett and Mount, 1967), the age at exposure (Lynch et al., 1976), the severity of the cold (Bateman and Slee, 1979), and the particular conditions of exposure (isolated or grouped, availability of nesting material) (Heroux, 1970). Cold exposure always stimulates an increase in metabolic rate. This is usually accompanied by an increase in food intake (Bateman and Slee, 1979; Barnett and Little, 1965), oxygen consumption (Barnett, Coleman and Manly, 1959) and haemoglobin levels of the blood (Maclean and Lee, 1973).

Often there is an increase in weight of organs such as heart, kidney, liver and intestines (Barnett and Scott, 1963; Barnett and Widdowson, 1965). The common pattern observed on exposure to cold is an initial decrease in body weight followed by a gradual increase if conditions are not too severe (Barnett, 1965). Behaviour also is affected. Mice build elaborate and well insulated nests in the cold and activity is often reduced (Wolfe and Barnett, 1977). But if the animal is kept isolated and without bedding there are often pathological consequences, and the animal cannot then be considered to be cold adapted (Heroux, 1970).

Changes in the insulative properties of skin or hair are of little value in an animal as small as a mouse. Insulation is proportional to thickness, regardless of the size of the animal. The small changes in hair length that a mouse could bear would do little to improve its insulation against the cold. The hair of inbred laboratory mice, however, does grow longer in the cold, but the skin is thinner (Barnett, 1959).

In summary, the primary ontogenetic responses of a mouse to cold involve increases in metabolic rate with associated organ changes and changes in behaviour, particularly nest building.

The patterns of adaptation to cold over many generations include the adaptive changes of the whole population over time which would involve changes in genetical composition. From this perspective the effect of cold on reproduction is an important consideration. Pregnancy and lactation make heavy demands on the mouse in addition to the need for greater heat production in the cold.

In a natural situation, cold, although important, is only one of a complex of interacting factors to which the mouse needs to adapt. To single out the effect of cold, laboratory procedures are necessary. Over a period of 20

years, Barnett and his co-workers have looked at the patterns of long term cold-adaptation by inbred and hybrid laboratory mice and by wild mice (reviewed by Barnett, 1965, 1973). It is from this extensive research that the present project originated.

The responses of mice to cold varied between strains, particularly between wild and laboratory mice but some general points can be made. Cold delays the onset of breeding and parturition intervals are initially lengthened (Barnett and Little, 1968). This may be due in part to raised thyroid hormone levels in the cold which in turn suppress serum gonadotrophin concentration (Narzian and Piask, 1976). Hence, fewer young are produced in a given time in the cold. If, however, the mice are allowed to breed to the end of their reproductive life the story is not necessarially the same. A cold-adapted inbred strain, A2G/Tb, kept for their full breeding span, produced the same total number of young as did their counterparts in the warm. Reproduction in the cold-adapted mice began later but lasted longer (Barnett, 1961). This did not however, apply to another inbred strain, C57BL/Tb, (Barnett,1961), nor to wild mice (Barnett, Smart and Stoddart, 1971). Infant mortality is initially high in the cold, but there is a gradual improvement over generations (Barnett, 1961). This pattern was similar for all strains tested.

The effect of cold on the growth of young differs between mouse stocks. The young of wild mice in the cold are heavier at 3 weeks than those of controls in all generations, whereas the young of the cold-adapted laboratory mice are lighter at 3 weeks than those of controls (Barnett and Neil, 1972). Wild rats, too, respond to cold differently from laboratory rats (Heroux, 1963).

In general, most mouse stocks improve in reproductive performance over the generations in the cold.

Even some inbred mice showed a decline in nestling mortality. Changes in the genetically heterogenous stocks such as wild mice and hybrid laboratory mice are presumed to be, at least partly, due to genetical change. Hence, when the cold-adapted stocks are returned to the warm environment they would be expected to differ in reproductive performance from the controls.

Although, in the cold, the cold-adapted wild mice were generally inferior in reproductive performance to those kept in the warm environment, when they were returned to the warm they were far superior to the controls in all aspects of reproductive performance. In contrast, cold-adapted hybrid laboratory mice when returned to the warm did not differ from the controls except that their young were heavier. Cold adapted inbred mice(A2G/Tb) which, in fact, had a lower nestling mortality in the cold than the controls, were also returned to the warm. Their reproductive performance quickly reverted to the control level. So again there were differences in the responses of different stocks to long-term cold exposure. These differences were not due only to differences in inbreeding (Barnett, 1973).

2.2.2. Analyses of maternal effects.

Nearly every character in a mammal may be influenced by the maternal environment. Only when the character shows variation can the maternal effect be recognised and investigated (McLaren, 1962). Differences in maternal influence are a result of alterations in the mother's environment and differences in genetical constitution. The young also influence the mother at all stages of development. Variations in their influence is also due to differences in genetic constitution and alterations in their environment. There is a continuing interaction between mother and young and the component influences are difficult to disentangle.

McLaren (1962) classifies maternal effects as cytoplasmic, uterine or milk effects, and, orthogonally, according to the type of alteration of the mother - genetical or environmental. She also outlines the experimental methods available for their analysis: egg or embryo transfer; cross-fostering at birth or reciprocal mating.

Apart from the extensive examination of toxicological effects on the pre-natal environment, maternal effects have been documented for a variety of characteristics including: brain development and behaviour (Flandera and Novakova, 1974; MacCarty and Southwick, 1979; Bresler et al., 1975; Ressler, 1962, 1966); number of lumbar vertebrae (Russell, 1948); susceptibility to spontaneous mammary cancer (Muhlbock, 1952; Gruneberg, 1952); Down's syndrome in human beings (Penrose, 1934); tail rings (Law, 1939); and growth (see below). Investigations into maternal influences on growth and reproductive performance are of particular relevance to the present study.

The questions explored by most investigators have been: what is the relative importance of pre- and postnatal maternal effects on growth; what is the scope of maternal effects on growth at different ages; and what is the role played by maternal effects in selection experiments. When a population is subjected to selection, say, for increased body weight, part of the increase is presumed to be due to 'direct genetic' changes in the animals and part to an 'indirect genetic' effect via improved nursing ability of females which may be correlated with increases in weight.

Variation in the prenatal maternal environment has been shown to influence the birth weight of young. Larger mothers tend to bear larger young irrespective of the genotype of the young. This has been demonstrated: with horses (Walton and Hammond, 1938); with cows (Dickinson,

1960); with rabbits (Venge, 1950); and with mice (Brumby, 1960). Brumby, however, found that a maternal environment which enhances fetal growth depends on factors other than body size. The maternal environment of the unselected mice in his experiment was superior to those selected for large body size. Differences in the prenatal maternal environment do not always influence growth. Al-Murrani and Roberts (1978) found that prenatal maternal effects, analysed by cross transfer of fertilized eggs, were not important in their large and small strains.

The growth rate of mice from birth to about 12 days is often assumed to be determined by the postnatal maternal environment. In fact, the weight of the young at 10 or 12 days is often used as a measure of the mother's lactation (Bateman, 1954; Nagai, 1971). This view has, in the main, been supported by experimental analysis. A large proportion of the variation in the 12 day weight of mice has been found to be due to postnatal maternal influence: 80% (Young, Legates and Farthing, 1965); 71% (Cox, Legates and Cockerham, 1959); 68% (Rutledge et al., 1972); 65% (Nagai, 1971) and 56% (Eisen et al., 1970). Others, however, have found uterine, genetical or a combination of both effects to be a greater influence (Brumby, 1960; Bateman, 1954; Moore et al., 1970). Wirth-Dzieciolowska (1975) showed that postnatal maternal effects observed in 6 inbred strains, varied according to the strains used.

Whatever the maternal influence at 12 days the effect declines once the young begin to eat solid food (Eisen and Roberts, 1981; Young, Legates and Farthing, 1965). Between 14 and 20 days of age the weights of mice are determined as much by genetic constitution as by milk source (Butler and Metrakos, 1950). Similarly, Stanier and Mount (1972) found that the growth rate of large line mice, suckled by small line mothers, increased after day 15.

The persistence of maternal effects into adulthood varies between strains and according to the initial magnitude of the effect. Maternal effects, evident at birth, found in reciprocal crosses between cows, disappear by the time the calves are 1 year old (Dickinson, 1960). But the differences between the F1 hybrids of reciprocal crosses between Shetland and Shire horses persist for the rest of their lives (Walton and Hammond, 1938). The persistence of maternal effects in mice varies greatly between strains and experiments. In reciprocal crosses used by Chai(1956) maternal influences accounted for one quarter of the total variance in weight at 60 days of age. Young, Legates and Farthing (1965) found that 16% of the variance in 56 day weight of their mice could be accounted for by postnatal maternal influence. Nagai, Bakker and Eisen (1976) also found persistant postnatal maternal effects on weight but not on post-weaning weight gain. Differences in maternal environments between 2 strains are often not symmetrical. Al-Murrani and Roberts (1978) found that postnatal maternal effects accrued mostly from the inadequacy of small mothers rearing large offspring: small offspring were hardly affected by the type of mother.

Experiments on maternal effects are not often followed through to later generations. Young, Legates and Farthing (1965), although they found large maternal effects on growth of the fostered young, did not find any transfer of the maternal effect. The reproductive performance of the fostered mice was unaffected by the type of mother who reared them, as was the weight of the young of the fostered mice. But Bresler et al., (1975) observed a grandmaternal effect on learning deficits in rats.

Evidence for the accumulation of maternal effects affecting growth and reproduction, comes from the work of Barnett on the adaptation of inbred strains to cold.

Highly inbred strains presumably have little room for further genetical change. After their introduction to a new environment they should remain stable. But after transfer to a cold laboratory there was a decline in nestling mortality in strains A2G and C57Bl over a number of generations (Barnett, 1961). The nestling mortality of controls kept in the warm changed little. No other aspect of reproductive performance changed in the inbred strains kept at $-3^{o}C$ in contrast to the changes observed in the random bred and wild stock. Also when A2G mice were transfered back to the warm after many generations at $-3^{o}C$ their nestling mortality was indistinguishable from the controls; the change in nestling mortality was not permanent which would be expected if it was genetically determined.

Subsequent experiments showed that the cold adapted inbred strains were superior in growth (mostly fat) and maternal performance to mice of the same strain newly introduced to the cold (Barnett, 1965). This was confirmed in fostering experiments; but the quality of the young was also shown to be important (Barnett and Neil, 1971). The important maternal effect in these experiments was the prenatal effect which was evident in the survival rate of the young of fostered mice.

Although other factors may explain the improvement in performance of the inbred strains after many generations at $-3^{o}C$, given other evidence the cumulation of maternal effects seems to be the most plausible.

2.2.3. Adaptability of mice: relevance of laboratory studies.

Mice are very successful colonizers with a nearly world-wide distribution. They live in diverse climatic conditions and through varying degrees of commensalism to an existence independent of man. Mice have lived in cold meat stores at $-10^{o}C$ (Laurie, 1946) and on islands about

or below the Antarctic convergence (Berry et al., 1978; Berry et al., 1979). The survival of mice for 150 years on an island with a climate such as that of South Georgia is a remarkable feat for so small a mammal (Berry et al., 1979).

What are the reasons for the house mouse's success? Mice are genetically very variable (Berry, 1977) and appear to have mechanisms for retaining variability (Rice and O'Brien, 1980). The reproductive cycle, which is very short and has no luteal phase in the absence of copulation, is thus specialized for maximum reproduction with no time or materials wasted; it is extremely opportunistic compared to the cycles of most other mammals (Conaway, 1971). The importance of the pheromonal system of mice in promoting successful colonization has been reviewed by Bronson (1979). The pheromonal system allows 'the avoidance of pregnancy before dispersal while promoting rapid ovulation once a home is established and the probable success of pregnancy is assured'. In addition to the opportunism of the reproductive ecology of mice, the physiological adaptability of mice, coupled with appropriate behaviour, enables them to survive in many extreme conditions (Barnett and Mount, 1967).

Making generalizations about adaptive responses of different mouse populations is difficult because of the possible importance of founder effects in determining the characteristics of each individual population (Berry et al., 1978). The genetical composition of mice from islands with similar environments, Maquarie and Marion Is, is more distinct from each other than either is from most British samples (Berry et al., 1978). Nonetheless, by comparing the phenotypic characters of many populations from different climates some generalizations can be made (Berry et al.,1981). Mice from cold climates tend to be larger, and to have relatively shorter tails and more brown fat, than those from warm equable islands. Mice

from Enewetak Atoll also have relatively smaller hearts and kidneys than mice from colder regions. Yet, although haemoglobin concentration increases in winter in Australian wild mice (McLean and Lee, 1973), haematological characteristics are similar for all populations (Berry and Jakobson, 1979). The warm populations are characterized by high genetical variation with no changes in heterozygosis or allele frequency with age; whereas populations living in cold regions had different allele frequencies at different stages of the life cycle. This 'provides strong presumptive evidence that cold stress may be an agent of natural selection' (Berry et al., 1981).

The conditions of mice in a laboratory differ from those of wild populations in many ways, including continuous food supply, absence of predators, cramped and boring housing, reduced social interaction and constancy of climatic factors such as temperature and humidity. Many problems of adaptation can, however, be analysed only by experimental manipulation, particularly the distiction between ontogenetic adaptation and the adaptation of whole populations over time.

For example, relative tail lengths of mice from cold areas are shorter than those of mice from warm areas (Berry et al., 1981). Laboratory studies reveal that tail growth is a direct response to temperature during an individual's lifetime (Harrison et al., 1959) Similarly, the increase in kidney weight in a cold environment is part of the immediate metabolic response of the individual mouse (Barnett and Scott, 1963). In contrast, higher body weight in a cold environment, although there may be some effect of ontogenetic adaptation, is principally a response occurring over many generations (Barnett et al., 1975).

Analysis of maternal effects, and their possible role in the adaptation of mice to new environments, would,

of course, be impossible without experimental manipulation.

The artificial nature of laboratory experiments must, however, be kept in mind when extrapolating findings to naturally occurring populations. 'Adaptability cannot be regarded as an isolated event for a particular organ, tissue or characteristic - it is a result of the integration of the whole phenotype' (Berry and Jakobson, 1975). Accordingly, Jakobson (1978) measured a range of phenotypic traits in a mouse population and found that no character on its own showed a significant correllation with winter survival but that a multivariate analysis was highly predictive. He writes: 'The survival of a mouse will result from the interaction of many facets of its biology with its environment: in nature it is rare for either of these to remain constant from year to year'.

3. MATERIALS AND METHODS

3.1 General Methods

3.1.1 Mice

The original mouse poulation was trapped at various poultry farms near Piallago, Austalian Capital Territory. The mice were then transferred to a laboratory kept at 21 C and 3Ø pairs were mated. The offspring of these pairs (generation 1) were mated and transferred to two constant temperature rooms: 15 pairs each to rooms kept at 23° C and 3° C respectively. At least one offspring from every fertile pair of trapped mice was represented in each environment. Thus the genetical composition of the two founding populations as similar as possible. The two populations were bred concurrently as outbred closed colonies for a further eleven generations. At least 15 pairs were mated in each generation.

In all other respects, choice of mice for breeding was random.

These mice provided the stocks for experimental manipulation. For simplicity the mice in the warm are called 'controls' and the those in the cold, Eskimos. An additional group of mice were trapped at the same poultry farm in March of the following year. These mice are referred to as 'trapped'.

An additional 6Ø mice were trapped at one of the poultry farms in the autumn of the following year. These mice are reffered to as 'trapped'.

3.1.2 Housing and maintenance

Mice were housed in standard black plastic tubs (26x2Øx13cm) with wire lids. Water and food (Mecon rat cubes) were provided at libitum. Bedding and nest material consisted of wood shavings and about 5 g non-absorbent cotton wool. Tubs were changed weekly except when a litter younger than 1Ø days was present.

Juveniles were housed as litter groups until aged 5 weeks and thereafter as like-sex litter groups.

3.1.3 Recording reproductive performance

Breeding pairs were mated at 6 to 10 weeks of age and kept for 23 weeks from mating or until their last litter born in that period was weaned.

During the weekly tub changing pregnant females were noted. Subsequently they were checked every day, in the morning, for the presence of a litter. This checking was done with a minimum of disturbance, often without removing the wire lid. If a litter was present, the nest was carefully opened and the pups counted without handling, and the nest quickly closed. If the pups were to be weighed they were lifted out of the nest by fingers rubbed in the nest material, placed on the balance and quickly returned. Juveniles were removed from their parents at three weeks of age, counted and weighed.

Records were kept of the date of birth of litters and number and weight at birth and at weaning.

3.1.4 Weighing and measuring

3.1.4.1 Mice

Mice were weighed on a Sartorius 3704 electronic balance to the nearest 0.1g except for pups at birth which were weighed to the nearest 0.01g.

Mice were measured to the nearest millimetre. Body length was measured as the distance from the tip of the nose to the anus and tail length from anus to tip of the pitella.

3.1.4.2 Organs

Kidneys and adrenals. The organs were trimmed of fat and other tissue and weighed on a Mettler balance to the nearest 0.01mg (adrenals) or 0.1mg (kidneys).

Stomachs of 10 day old mice. The stomachs were removed by snipping the end of the esophagus and at the pylorus. They were weighed to 0.1mg on a Sartorius 2402 pan balance. The stomach was then cut and the milk curd

removed. The curd usually came out cleanly; but if not the stomach contents were removed by scraping with the blunt end of scissors. The stomach was then weighed again.

3.1.5 Statistical analysis

Unless otherwise indicated all analyses were performed on a Univac computer using SPSS statistical packages.

Descriptive statistics were performed for every class and measure. Statistics computed included mean, standard deviation, standard error, variance, skewness and range.

Two-tailed Students t-test was used for comparisons of means. If samples had unequal variances an approximation to t was computed.

Two and three way analyses of variance were performed to analyse the effects of independent factors such as, temperature, true parent, foster parent, on the various measurements. A fixed effect or linear hypothesis model was used.

Bivariate statistics included correlations between body weight and organ weight, body fat and body water. Descriptive scattergrams were drawn and Pearsons correlation coefficient calculated. Intercept and slope for simple linear regression were also computed. Simple regression coefficients were calculated for the regression of various measurements on generation and for the regression of body weight on litter size. A multiple regression model was computed for the relationship between pup weight, milk intake and maternal weight using Genstat procedures.

Relative growth was calculated using the equation

relative growth = 100(LN(weight1 - weight0))/10

Relative organ weights were calculated on a G per 100 G basis. Although organ weights do not change

arithmetically with body weight, over a small range of body weights there is often a strong linear correlation between organ weight and body weight. In this case it is acceptable to compare relative organ weights of groups differing in mean body weight. Kidney weights were compared in this way. Adrenal weights, however, were not correlated with body weight so absolute values were compared.

A general problem in many of the analyses was whether the litter or the individual young should be the replicates. A one way analysis of variance was carried out on all measurements made for every class. In every case the between litter variance was always higher than the within litter variance. Hence, it appeared that the litter should be regarded as the replicate unit rather than the individual young. So in every analysis unless otherwise indicated, the mean value for each litter was used as a replicate. There was a change in the F ratio (between litter variance/within litter variance) with age. The following table gives the F ratios from a one way analysis of body weight for each age group of generation 10 mice in the cold. The pattern of change is representative of all other classes.

Age, days	F ratio	P
0	15.0	0.001
10	38.0	0.001
21	23.5	0.001
42	12.5	0.05
70	7.8	0.1
112	9.1	0.05

After birth there was a sharp increase in the F ratio by 10 days followed by a more gradual decline to a fairly steady level after 42 days of age. After 10 days of age the within litter variance becomes greater but it

is always smaller than the between litter variance. Although the F ratios were still significant after 21 days in this particular example it was not always so. Individual animals were used as replicates from the age of 42 days to ensure sufficient number of replicates.

When litter means were calculated male and female weights were combined. There was no difference between males and females at birth or 10 days but at 21 days when all classes were combined males were heavier (8.2±0.03) than females (8.0±0.03) ($P < 0.001$). However all classes had similar sex ratios at 21 days so the combining of male and female weights would not affect the comparison of classes.

3.2 Specific Methods

3.2.1 Cross fostering

Cross fostering was performed at generation 6 (fig 2). This required transferring mice between temperatures at generation 5; as a result the mice used as foster parents had been in one environment throughout their lives. For the fifth generation 4Ø pairs of control mice were mated. Of these, 2Ø remained in the warm room and 2Ø were transferred to the cold room. Similarly, 4Ø of Eskimo mice were mated; 2Ø pairs remained in the cold and 2Ø were transferred to the warm. For the sixth generation more pairs than in previous generations were mated to ensure sufficient simultaneous litters available for cross fostering. There were thus 4 classes of foster parents (Table A).

Table A. No. of mated pairs, generation 6

	AMBIENT TEMPERATURE	
	WARM	COLD
control	26	24
Eskimo	26	3Ø

Hence in each temperature there were four classes of fostered mice: two between-class and two within-class. The latter represented controls. Numbers of successful fosterings per class varied (Table B).

Table B. No. of successful fosterings
(in parenthesis total no. of attempted fosterings)

	WARM		COLD	
	FOSTER PARENT		FOSTER PARENT	
	control	Eskimo	control	Eskimo
con	17(18)	16(19)	13(2Ø)	13(19)
YOUNG				
Esk	15(15)	15(16)	14(17)	11(16)

Only second, third or fourth litters were used for cross fostering. Pregnant females were checked daily, in the morning, for the presence of a litter. When two or more litters were born on the same day cross fostering was carried out as follows. (Occasionally some mice were used as fosterparents who had littered the previous day.) The litter was removed from the nest, reduced to 5, weighed and placed in its prospective foster parents' nest.

The litter was painted with the foster mother's urine with a soft brush. The litter then remained in the nest for 2Ø min before the foster parents were returned. The cage was thereafter left undisturbed for 1Ø days.

When the pups were 1Ø days old they were sexed and weighed; and the family was transferred to a clean cage. Pups were removed from their foster parents at 21 days and again weighed.

Sufficient fostered young were kept for mating at 6 to 1Ø weeks of age, and extra males were kept for weighing at 1Ø weeks and 16 weeks. Ten pairs were mated in each fostering class and reproductive performance recorded as above (with the addition of 1Ø day pup weights). At 16 weeks males were weighed, body and tail length measured, kidneys and adrenals weighed and body composition analysed (section 3.2.4 below) The males of the mated pairs were killed at the end of the reproductive period and similarly treated.

3.2.2 Reciprocal mating

Mice of the 1Øth generation were used for reciprocal mating (Fig. 2). Transfers were made at the 9th generation. 3Ø pairs of control mice were mated, of which 15 pairs remained in the warm and 15 pairs were transferred to the cold. Similarly, 3Ø pairs of Eskimo were mated, of which 15 remained in the cold and 15 were transferred. Reciprocal matings were made only in the cold environment. The two classes of mice, Eskimo and control, were mated within and between classes reciprocally. There were 15 pairs in each of the four resulting classes. (Table C).

Table C. Nomenclature for young of reciprocally mated pairs

		MOTHER	
		control	Eskimo
FATHER	control	control	hybrid with Eskimo mother
	Eskimo	hybrid with control mother	Eskimo

The reproductive performance of these pairs was recorded as above (but including 10 day pup weights). Pups were sexed, and were weighed individually at birth, 10 days, and 21 days. 20 litters from each class were kept after 21 days. Males and females were weighed at 6 weeks and males only at 10 and 16 weeks. Males aged 16 weeks were also measured, their kidneys and adrenals were weighed and their body composition analysed. 10 pairs from each class were mated and their reproductive performance recorded. At the end of the reproductive period the adult males were killed, weighed and measured, their kidneys and adrenals were weighed and their body composition analysed.

3.2.3 Milk

The lactational performance of three classes of mice was measured in the 10th generation. Controls were studied in both environments (1st gen in the cold) and Eskimos only in the cold. Lactating mothers were used 23 weeks after mating, when they were rearing their 5th to 7th

litters. The litters were reduced to five at birth. When the litter was 1Ø days old the mother was either milked or injected with a radioactive isotope for calculation of 24-h milk production.

3.2.3.1 Milking

On the day of milking, the mother was separated from her pups at 1ØØØ hrs. At 1345 hrs she was injected with Ø.5 IU Oxytocin (Grade III synthetic aqueous solution, Sigma). At 14ØØ hrs the mother was anaesthetised with ether/air and milked with an apparatus based on that designed by Feller and Boretos (1967) (Fig. 6.1). Milk was drawn into collection vials by light suction. The procedure was repeated with equivalent volumes of cows' milk to control for evaporative losses. All nipples were milked twice, in order, from rear to front. At least Ø.5 ml was obtained in each sample. Milking was always finished within 2Ø min. Samples were labelled and immediately stored in a refrigerator at -4Ø^{o} C until analysed. The maximum storage period was 3 months. Equivalent volumes of cows' milk were also frozen.

3.2.3.2 Milk composition

32 samples of mouse milk, each from a different female, and 1Ø of cow's were analysed all at the same time. Measurements were made of specific gravity, total solids fat and protein.

For specific gravity and total solids the samples were warmed to 37^{o}C to liquefy the fat. Milk was taken up in a weighed 5Øul Microcap (Drummond) and weighed to the nearest Ø.1 mg. Specific gravity was the weight of the milk (mg) divided by 5Ø.

The milk was then expelled on to a weighed aluminium dish. The capillary tube was rinsed with distilled, deionized water and the rinsings were added to the dish. The sample was dried at 5Ø^{o}C in a vacuum oven

for 1Ø h, cooled over silica gel and reweighed.

$$\text{Total solids} = \frac{\text{dry solids,mg}}{\text{milk,mg}} * 1ØØ$$

For estimates of fat and protein 5Øul samples were taken in triplicate. The value for each sample was calculated as the mean of three measurements.

Fat was extracted by a method based on that of Bligh and Dyer (1959). The sample was placed in a 1Ø ml graduated centrifuge tube. To this was added 1.Øml methanol, Ø.5ml chloroform and Ø.35ml water. The tube was shaken for Ø.5 min. A further Ø.5ml chloroform was added and the shaking repeated. Finally Ø.5ml water was added and the tube was again shaken, and spun at 1Ø,ØØØ rpm for 3 min. The mixture separated into three phases. The top phase was methanol and water containing carbohydrates and minerals, the central layer was a solid white protein precipitate, and the bottom layer contained the chloroform and fat. The methanol layer was removed with a Pasteur pippette and discarded. The chloroform containing the dissolved fat was removed by pushing aside the protein precipitate with a Pasteur pipette and removing the bottom layer. The chloroform was dispensed on to a pre-weighed aluminium dish and placed in a vacuum oven at 5Ø°C for 1Ø h. Each sample was cooled over silica gel and weighed to the nearest Ø.1mg. This gave the percentage of fat.

1 ml 1M NaOH was added to the protein pellet in the centrifuge tube, mixed, then heated in a boiling water bath for 1Ø min. Standard solutions of BSA (Fraction 5 BØ21 Commonwealth Serum Laboratories) were made up and similarly treated. Protein was estimated by the method of Lowry (Lowry et al.,1951). Samples were read against a reagent blank in a Beckman DB spectrophotometer. Protein values for the samples were read off a standard curve.

3.2.3.3 Milk production

3.2.3.3.1 Methods of assessing milk production

The standard techniques for estimating the milk production of mice have been, until recently, direct milking and test weighing.

Direct milking is a coarse method for the small amounts of milk involved, and experimenters vary greatly in their ability to extract mouse milk.

Test weighing usually involves a pre-testing fast of 4 to 12 hrs. The pups are then weighed immediately before being allowed to suckle and again at the end of an arbitarily defined period. Even when urinary and faecal losses are controlled for, this method has disadvantages: for example, the rate of secretion in the mammary gland probably begins to decline after 4 hrs of non suckling (Linzell, 1972). But the major disadvantage of the method is that it is unrelated to the normal nursing pattern. Mice do not have periods of feeding at regular intervals. Pilot observations on ten nursing mice indicated that, during the day, the young are attached to the mothers teats almost continuously. Sucking is sporadic and irregular. Similarly Cross (1977) found that rat pups remain attached to the teats for long periods but milk removal is limited to brief intermittent milk ejections occuring at intervals of 5 to 10 minutes. Correspondingly, whatever time of day the mouse pups in the present study were killed their stomachs were always full.

Hence, when groups are compared by test feeding, differences in lactational performance due to differences in the behaviour of pups or mother would probably not be apparent. The ideal method of calculating milk production would be to measure the milk yield over the whole lactation period, or at least for 24 hours.

In the last 10 years methods have been developed to measure milk yield by using radioactive isotopes (Yates et al. 1971; Romero et al. 1975; Baverstock and Elhay, 1978; Green and Newgrain, 1979; Rath and Thenen, 1979).

3.2.3.3.2 24-h milk intake with Tritium

The method used here was based on that of Rath and Thenen (1979). On day 9 the pups were individually marked with Durafur (ICI Australian Petrochemicals) and the mother weighed. On day 10 at 1000 hrs the pups were weighed and the mother injected with 25 uCi tritium (Amersham) Food was now still supplied but no water. After 1 h a 10 ul blood sample was taken from the mother's tail and counted. Tritiated drinking water was prepared to 140% of the activity of the mother's blood (usually about 1.8 uCi/ml). This was to keep the activity of tritium in the milk constant over the 24-h period. The mother and litter were then left for 24 h in the appropriate temperature.

On day 11 at 1000 hrs the pups were killed and triplicate 10-ul samples of blood taken from the femoral artery of each. Similar samples were taken from the tail of the mother. Each sample was placed in 10 ml Scintillation Fluid (PCS, Amersham) and counted on a Beckman L5 100C scintillation counter. A quenching curve was drawn by using concentrations of blood from 1 ul to 50 ul of known radioactivity. The disintergrations per min (dpm) for each sample was calculated by multiplying the counts per min (cpm) by the efficiency determined from the quenching curve. Milk intake for each pup was then calculated by the equation of Rath and Thenen(1979).

$$\text{milk intake} = \frac{\text{dpm/ul pup blood} * \text{body water of pup}}{\text{dpm/ul maternal blood} * \text{water fraction of milk}}$$

The body water of the pup was determined from a regression curve based on the body composition of mice aged 1Ø days (6.3). The water fraction of the milk was determined from an analysis of milk (Section 3.2.3.2).

Despite some good features this method has several sources of error. There is no estimation of tritium efflux in pups. Urinary, pulmonary and cutaneous losses are likely to be substantial over 24 h, and failure to account for them would lead to underestimates of milk intake of up to 2Ø%. No account is made of metabolic water produced from milk solids in the pups. This again would lead to underestimating milk intake.

3.2.4 Body composition

Measures of total body fat and body water were made on eviscerated carcasses. Mice were killed with chloroform, weighed and eviscerated (liver, gastrointestinal tract, kidneys and adrenals were removed). The carcasses were stored in plastic bags at -40°C until analysis.

WATER. The carcass was chopped into small pieces with a scalpel and placed on a weighed petrie dish in a semi vacuum oven at 40°C. The carcass remained in the oven until weight was constant. This usually took 2 days. The dish containing the dried carcass was removed to a desiccator before weighing to eliminate condensation on cooling.

$$\% \text{ water} = \frac{(\text{wet wt}) - (\text{dry wt})}{(\text{wet wt})} * 100$$

FAT. Determination of fat content was based on the method of Bligh and Dyer (1959). To the pieces of dried carcass was added 40ml water, 50ml chloroform and 100ml methanol. This mixture was homogenized in a Waring blender at low speed for 1 min. 50ml water was added and the mixture was homogenized for 30 sec. Proportions were then 1.8:2:2 which was necessary for separation into two phases - water and methanol on the top phase and chloroform containing the dissolved fat in the bottom phase. The phases were evident after the mixture was filtered through Whatman's No 1 filter paper in a Buchner funnel under a slight vacuum. The residue was pressed down with a beaker until it was dry. It was necessary to rehomogenize the filter paper and residue in 50ml chloroform for 1 min to ensure quantitative results. The second mixture was filtered and the residue discarded. The filtrate was poured into a separating funnel and allowed to settle for

60 min. The chloroform layer containing the dissolved fat was collected and the methanol layer discarded. The fat was extracted by redistilling the chloroform in a rotary evaporator. Finally the fat was weighed.

$$\% \text{ fat} = \frac{(\text{fat wt})}{(\text{wet wt})} * 100$$

Once the fat weight was determined the fat free carcass weight was calculated.

$$\text{fat free carcass wt} = \text{wet wt} - \text{fat wt}$$

%water was recalculated on the fat free carcass weight.

$$\% \text{ water} = \frac{[\text{fat free carcass wt} - (\text{dry wt} - \text{fat wt})]}{\text{fat free carcass wt}} * 100$$

Determination of fat content of 10 day old mice was the same as for adults except that only half quantities of reagents were used.

3.2.5 Nest temperatures

The nest temperature of eskimos and 'controls' was measured in both temperatures at generation 6.

Temperatures were recorded with a Cormark electronic thermometer (Type 1604 Cr/Al) attached to a flat bed pen recorder (Rikadenki B-34) using 50mv and full scale deflection. All measurements were made when the pups were 2 days old. Litter size varied from 4 to 10.

A standard procedure was followed. The cage top was lifted and the parents removed from the nest. The probe was placed immediately in the centre of the pups, the

opening in the nest was repaired, and the temperature was recorded after 5 min. The probe was next placed on the outside of the group of pups, touching a pup, the opening in the nest was again repaired, and the temperature recorded after a further 5 min. The probe was next placed 2 cm away from the pups in the nest space and the same procedure was followed. In addition, some temperatures were recorded continuously for 20 minutes with a probe in the centre of the pups.

3.3 General Experimental Conditions

3.3.1 Levels of inbreeding.

The mice were always outbred, and at least one offspring from each pair that weaned young was used as a parent in the next generation. Nonetheless some inbreeding was inevitable since the populations were small and closed.

Inbreeding can depress reproduction and affect general 'vigour' (Falconer, 1960). Since a comparison of reproductive performance between the two stocks, warm and cold, was an essential part of the present study it was important that inbreeding was not a confounding influence.

Wright's inbreeding coefficient was calculated for each generation in both temperatures by the co-ancestry method of Cruden (1949) programmed by Li and Roderick (1970). The initial generation (trapped mice) was assigned an inbreeding coefficient of zero. The mean inbreeding coefficient of both Eskimos and controls increased over the generations as expected (Fig 3.1). After the third generation inbreeding among the Eskimos was greater than among the controls, but the coefficients of generation 9 Eskimos were similar to those of generation 10 controls.

The inbreeding coefficients were fairly high but were within the same range as those estimated for feral mouse colonies in South Australia (0.17 - 0.27) (Greg Kirby, pers. comm.). Although the coefficients were nowhere near the levels associated with full or half-sib mating, depression of reproductive performance could be expected. Lynch (1977) found that litter size was depressed at levels of inbreeding less than 20% in wild mice. However it may be that little genetic variation is lost by breeding in a closed population. Rice and O'Brien (1980) found that the level of heterozygosis of Swiss mice, bred from 9 original mice for 175 generations in closed populations, was not much lower than that of feral

mouse populations.

Whether feral populations are themselves to some degree inbred is a matter of debate. It is unlikely that wild mice populations have no breeding structure and form a complete panmitic unit (Anderson et al., 1964). It is also unlikely that they are made up entirely of discrete inbred units with little migration, although this situation might exist in some cases for a short time (Petras, 1967). Breeding structure changes with changes in the fortunes of a population (Myers, 1974). Over a number of years there would probably be fairly extensive genetic mixing. Berry and Jakobson (1974), working with an island population for many years, found that 20% of individuals breed in an area other than the one they were born in.

It would appear from the work of Rice and O'Brien that domestic mice have mechanisms for maintaining variation. Rats, however, may not: Eriksson et al. (1976) found that the genetic variance of outbred and inbred laboratory strains of rats was much less than that of feral populations.

Probably, the mice of the present study, although somewhat inbred, did not suffer an appreciable loss of genetic variation.

3.3.2 Nest temperatures

The ambient temperatures of the two laboratories in which the mice were bred differed by about 20°C. Mice, however, build insulating nests and it was possible that the nest temperatures in the cold were not greatly different from those in the warm.

An attempt was made therefore to measure the temperatures of nests containing young litters. Such measurements present a number of difficulties. There are large variations, particularly in the cold, across the nest, temperatures fluctuate considerably when the parents

go in and out of the nest and also the age of the nestlings affected nest temperature (Barnett, 1956). Young mice are nearly poikilothermic until 7 days old. They gradually develop homeothermy and at about 17 days are fully homeothermic (Lagerspetz, 1966). To compare classes in the present study standard measurements were needed. These tended to be rather artificial (3.2.5).

Table 3.1 gives nest temperatures for both control and Eskimo mice in both environments. All nest temperatures were well above the ambient temperature hence the nests built by the mice in the cold environment insulated the nearly poikilothermic young to quite a large extent. Nests in the cold were always colder than those in the warm but there was no difference between mouse types in the same ambient temperature. Hence, as expected, mice reared in the cold were subject to colder nest temperatures than those in the warm. The nestlings in the cold were probably also subjected to greater fluctuations in temperature. The mid-nestling temperatures of some nests were measured for 20 min after the parents had been removed and the temperature usually remained fairly stable, dropping only a few degrees. No quantitative assessment of nest quality was made; but mice in the cold invariably had a thick woollen floor to their nests whereas in the warm the nestlings were often on the bare plastic tub or a thin covering of wood shavings.

Litter size had an effect on nest temperature. Litter size was positively correlated with nest temperature in both environments (Fig. 3.2), especially in the cold.

4. GRADUAL CHANGES AND COLD ADAPTATION

The general aim of the following experiments was to examine the structural and physiological changes that occur during the long-term adaptation of a house mouse population to cold.

4.1 Experimental Design

The experimental design is illustrated in Fig 2.2 Two stocks of house mice originating from the same population were bred concurrently in a warm (23°C) or a cold (3°C) laboratory for 12 generations. It was expected that both stocks would change. Changes in the warm ('controls') would represent adaptation to a laboratory environment and drift. Changes in the cold population ('Eskimo') would reflect adaptation both to the laboratory environment and to the cold conditions, as well as random effects.

Measurements were made of reproductive performance and other physical characteristics. In each generation total reproductive performance was assessed by recording number, size, and weaning weight of litters produced in a defined period (3.1.3). At the end of the breeding period the males were weighed, their body and tail lengths measured, kidneys and adrenals weighed (3.1.4) and finally carcasses analysed for total fat and water content (3.2.4). A group of 'trapped' mice were similarly treated (3.1.1). The weights of mice at three weeks (weaning) were also used as indicators of growth.

To assess the extent to which the two populations had diverged it was necessary to compare Eskimo and controls in the same environment. At generations 5 and 9 some Eskimo and control pairs were therefore transferred to the opposite environment soon after they were mated. The standard measurements described above were made on these transferred mice and on subsequent generations bred from them.

4.2 Changes Over Generations

4.2.1 Reproductive performance

At every generation at least 15 pairs were mated and kept for 6 months (Table 4.1).

The principal measurements of reproductive performance were the number and weight of young reared to weaning. The number of young weaned is the product of the number born and the subsequent pre-weaning mortality. The total number of young born is determined by litter size and the number of litters produced. The number of litters produced is a reflection of the delay from mating to the birth of the first litter and of subsequent parturition intervals.

Changes over generations are summarized in Table 4.2.

The reproductive performance of the controls remained fairly static over the generations, but the number of young born per pair and the weight of young at 3 weeks both increased slightly.

In contrast, the reproductive performance of the Eskimos improved in nearly every respect. The number of young born and percentage of young reared per pair both increased, and consequently so did the total number of young weaned. Litter size did not change progressively over the generations: most of the increase in number of young born was due to an increase in the number of litters (Table 4.1). This in turn was due mainly to the decrease in time taken to produce the first litter (Fig. 4.5) but partly also to shorter intervals between parturitions. After generation 5 an increasing percentage (up to 40%) of the parturition intervals of Eskimos were shorter than 26 days. The corresponding figure for controls remained about 20% (Fig. 4.4). Most conceptions of laboratory mice occur at the post partum estrus and the gestation time is subject to lactation delay; in the absence of concurrent lactation the interval is about 21 days, but if the current litter survives, the delay till birth is

proportional to the number of young weaned in the current litter (Barnett and Little, 1968; Fuchs, 1982). The higher percentage of shorter parturition intervals in later Eskimo generations were, however, not due to greater losses of whole litters or to smaller litters: the decline in parturition intervals began when losses of whole litters had declined (Fig. 4.2); and there was no change in litter size over the generations.

The weight of young of both classes at three weeks increased over the generations but the progressive increase in the weight of Eskimo young was steeper. The improvement in the reproductive performance of the Eskimo mice then included both an increase in the number of young weaned and an increase in their weight. Hence the biomass of the young produced rose even more steeply.

The improvement in the reproductive performance of the Eskimos was particularly notable considering the degree of inbreeding present (3.3.1). The inbreeding coefficients were high enough for inbreeding depression to occur yet the Eskimo stocks improved in those aspects of reproduction considered sensitive to inbreeding depression.

A comparison of the two colonies, each in its usual environment, shows the Eskimos to be initially inferior to the controls in most aspects of reproductive performance. There were more barren pairs (Table 4.1) and the fecund females produced fewer young (Table 4.4) due to initial delays in breeding (Fig. 4.3). The young that were reared were lighter at 3 weeks (Fig 4.5). The most stiking difference was in mortality rates (Table 4.4). Control mice always reared 90% or more of their young. Initially the Eskimo mice reared 50% of their young, then only 40% in the second generation. Thereafter there was a gradual decline in mortality, but Eskimos always reared a smaller proportion of their young than controls (Fig. 4.1). Most

of the pre-weaning mortality in the cold was due to losses of whole litters. After a peak in generation 2 there was a gradual decline in whole litter loss of Eskimo mice (Fig. 4.2).

There was no difference between controls and Eskimos in litter size at birth at any time (Table 4.3). In the cold environment, where there were always substantial losses of whole litters, there were differences in litter size at birth between those that were reared and those that died. The litters which did not survive tended to be smaller.

After generation 7, the reproductive performance of Eskimos, apart from the high infant mortality, was equal to and in some respects superior to that of the controls. In particular, by generation 7 Eskimos weaned as many young as the controls and these young were heavier (Fig. 4.5).

Litter size:

Reproductive measures in multiparous animals are complicated by the influence of litter size on pup weight as well as the effect of parity on both pup weight and litter size (Barnett et al, 1975; Barnett et al, 1971).

There were no differences in litter size between any classes (Table 4.3). The fewer litters per pair produced by the Eskimo mice in the first generation, however, meant that most litters weighed were of first to third parity compared to first to fifth parity of control mice. Table 4.5 gives correlations of 3 week weights with litter size and parity for each class. For the first generation there was no evidence of an effect of parity on three week weight; hence the weaning weights should not be affected by an unequal distribution of parities.

The results given in the table are difficult to interpret. In a few classes there was a negative correlation of 3 week weight with litter size and in even

fewer there was a positive correlation of of 3 week weight with parity. The effect of these two factors may not have been large enough to show up in such small classes where many other variables were acting to influence 3 week weight. A further complicating factor was that litter size was positively correlated with parity in some classes. However, even when partial correlation coefficients were calculated to control for (i) parity and (ii) litter size the situation was not clarified. Classes could not be grouped into either all Eskimo or all control since 3 week weight varied with generation for both stocks. Some generations, however, which were similar in three week weights were pooled. The effect of littersize on 3 week weight is described for generations 2-5 controls and generations 3-5 eskimos (Fig. 4.6). There was no consistent effect of litter size on the 3 week weights of Eskimos. The change in mean weights of control mice with litter size did, however, follow a rough pattern. Weights of litters smaller than 4 were low; there was a plateau between litter sizes of 4 and 7; litter sizes greater than 7 tended to weigh less.

4.2.2 Body measurements and organ weights

The gradual changes in body weight and dimension over the generations are described in Figs 4.7 to 4.10. Table 4.6 gives linear regression coefficients for measurements regressed on generations.

Trapped mice were lighter and smaller than both classes in the laboratories ($P < 0.001$). This was presumably an effect of conditions of captivity, especially continuous supply of immediately available food. After the third generation the Eskimo mice began to be heavier than the controls (Fig. 4.7); and by the ninth generation the Eskimo were more than 5 g heavier. The controls also increased in weight over generations, but at a slower rate. See Table 4.6 for regression coefficients.

Changes in body length followed a similar pattern (Fig. 4.8). The body lengths of the controls remained fairly constant over the generations, but those of the Eskimos increased after the 4th generation: by the ninth generation the Eskimo mice were more than 5 mm longer than the controls.

Eskimos consistently had shorter tails than controls (Fig. 4.9). Tail length of control mice did not change over generations but after generation 2 the tails of Eskimo mice progressively increased in length. Tail length evidently increased allometrically with body length since the ratio of tail length to body length, however, remained fairly constant at 75% (Fig. 4.10). The tail/body length ratio of controls oscillated about 90% over generations. Trapped mice had a ratio similar to that of the controls.

The changes in organ weights over generations are described in Figs 4.11-13. Table 4.7 gives linear regression coefficients for organ weight regressed on body weight.

The kidneys of the Eskimo mice were heavier than those of the controls from generation 1 onwards (Fig. 4.11). Kidney weight was highly correlated with body weight, more so with Eskimos than controls (Table 4.7); but when the kidney weights were expressed as a proportion of body weight, those of the Eskimo mice were still heavier than those of the controls (Fig. 4.12). Relative kidney weight was fairly constant over the generations for both classes. The relative kidney weight of the trapped mice was similar to that of the control mice.

Over the generations adrenal weight tended to decline (Fig. 4.13). There was a large decrease in adrenal weight of Eskimos at generation 5. After this generation adrenal weight remained fairly constant. The increase in weight and length of Eskimos also began at generation 5. The adrenal weights of controls were not

correlated with body weight but those of the Eskimos were negatively correlated with body weight (Table 4.7). Since, however, body weight increased over generations and adrenal weight decreased, the negative correlation could be due to a generation effect. Partial correlation analysis revealed that when the generation effect was controlled for there was no correlation between body weight and adrenal weight (Table 4.7). There was no consistent difference between the Eskimo and control mice in adrenal weight, although Eskimo adrenals tended to be heavier in the first 4 generations. Adrenals of trapped mice were heavier than both Eskimos and controls ($P < 0.001$).

4.2.3. Body composition

Findings on body composition are presented in Figs. 4.14-16.

Although figures are not available for all generations, some trends can be identified. The body fat of controls changed little over the generations. In contrast, after generation 4, the body fat of the Eskimo mice increased both absolutely and relative to body weight (Fig 4.14). Hence much of the difference in body weight between the two classes was due to fat. Nonetheless the lean weight of the Eskimo mice also increased over the generations, and so became superior to that of the controls (Fig. 4.15).

Percentage water was negatively correlated with fat in both groups (Fig. 4.16). This is a common finding. Eisen and Leatherwood (1981) have found that percentage water is a reliable predictor of percentage fat. Correspondingly, the percentage water of the controls remained fairly constant over generations but that of the Eskimos declined.

4.3 Comparison of Eskimos and Controls Each in the Same Environment

4.3.1. Generation 5 transfers

4.3.1.1. Reproductive performance

Reproductive performance was assessed for transferred mice as described in Section 4.2.1 and was compared with that of the indigenes. The results are presented in Tables 4.8-11.

The reproductive performance of the Eskimo mice transferred to the warm at generation 5 and that of the subsequent generations 6 and 7 resembled that of the controls in most aspects. Number of young produced and mortality rates were much the same (Table 4.9).

The controls transferred to the cold, however, differed from the eskimos in the cold. There were more barren pairs among the controls.

The most notable effect of transfer to the cold was the lengthening of the interval between mating and birth of the first litter (Fig. 4.3). This meant that the Eskimo mice produced more young than the transferred controls since more litters were produced during the assigned breeding period (Table 4.8). There was, however, no difference between the two classes in pre-weaning mortality (Table 4.9).

There were inconsistent differences in litter size between the two classes. In generation 5 control litters were larger than those of Eskimo but in generation 7 Eskimo litters were larger than those of controls (Table 4.1Ø).

There were many differences between classes in the weight of young at three weeks in each generation (Fig.4.17). To determine the contribution of temperature and mouse type to variation in three week weight two way analyses of variance were performed for each generation (Table 4.11).

In each generation both ambient temperature and mouse type had consistent effects on three-week weight. Eskimo young were always heavier than controls in both temperatures; but mice in the cold weighed less than those in the warm. In generation 5 temperature had by far the greater effect and there was also an interaction between temperature and mouse type: the effect of temperature on control mice was greater than on Eskimo; and the effect of mouse type was greater in the cold than in the warm. There was no such interaction in generation 6. In generation 7 the effect of temperature was much less; the Eskimos in the cold had reached the point, illustrated in Fig. 4.5, where they were heavier at three weeks than the controls in the warm. The young of controls in the cold meanwhile, were heavier in each successive generation and by generation 7 their three-week weights were no different from those of controls that had remained in the warm. There was, however, still a strong effect of mouse type, for Eskimos were still heavier; but there was an interaction: although Eskimos were heavier than the controls in the cold there was little difference between the two classes in the warm.

4.3.1.2. Body measurements and organ weights

Eskimo mice of generation 5, transferred to the warm at mating, were the same weight as the controls at the end of the breeding period; but they were were longer, and their tails were shorter (Tables 4.12-13). Generations 6 and 7 Eskimo (derived from the transferred mice of generation 5 and reared in the warm) resembled the controls in weight and length, but their tails were longer both absolutely and proportionally to body length (Table 4.12). The Eskimos of generations 6 and 7 in the warm differed considerably from the Eskimos that remained in the cold. Those in the warm were lighter ($P < 0.001$) and shorter ($P < 0.05$) and had longer tails ($P < 0.001$).

The controls transferred to the cold at mating, were the same weight and length as the Eskimos but heavier and longer than the controls in the warm. But generation 6 and 7 control mice which were reared in cold were much lighter and shorter than the Eskimos (Tables 4.12-13). They did not differ from controls that remained in the warm, except that their tails were shorter.

The parents of the transferred mice of generations 6 and 7 differed in the environments in which they were reared. The mothers of mice of generation 6 were reared until 8 to 1Ø weeks of age in their temperature of origin and then transferred to the new temperature where they remained throughout their breeding life. In contrast, the mothers of generation 7 were conceived and reared, and spent their breeding life entirely in the new temperature. Hence the environment (pre- and post-natal) provided by these two sets of mothers probably differed. Hence for generations 6 and 7, then, there were three probable sources of variation: (i)the ambient temperature in which the mice were reared; (ii)their genetical type (control or Eskimo); and (iii)maternal environment (generation 5 contrasted with generation 6 mothers).

Three way analyses of variance were therefore performed for body weight, body and tail length (Table 4.14). All three factors influenced variation in body weight and length. Mouse type had the largest effect: again, Eskimo mice were heavier and longer. Temperature was also an important source of variation, but there was an interaction between mouse type and temperature: the weights and lengths of the control mice were similar in each temperature, whereas Eskimos were heavier and longer in the cold than in the warm. There was also an effect of maternal environment: mice of generation 7 were heavier and longer than those of generation 6.

Temperature of rearing had by far the greatest effect on tail length. This was expected. Tail growth has

been shown to be a function of ambient temperature during growth (Harrison et al.,1959). There is a reduction in tail length in the cold for both inbred laboratory and wild mice (Barnett, 1965; Barnett et al., 1975). But mouse type also influenced tail length: Eskimos had consistently longer tails than controls. There was no evidence of an effect of maternal environment.

The findings on organ weights are presented in Table 4.15.

There were many differences in absolute kidney weights between classes, but these were primarily related to differences in body weight (see 4.2.2). The main influence on variation in relative kidney weight was temperature. Relative kidney weights in the cold were nearly always much higher than those in the warm. Barnett and Scott (1963) similarly found that relative kidney weights were consistently higher in the cold with all strains of mice tested. There was rarely any difference in relative kidney weights between Eskimo and control at the same temperature. The exception to these general trends was the generation 7 Eskimo class in the warm. These mice had relative kidney weights higher than those of any other class in the warm, and similar to relative kidney weights in the cold.

The adrenal weights of all classes were nearly always similar.

4.3.1.3 Body composition

Findings on body composition are presented in Table 4.16. There were no differences in body fat between controls and Eskimos in the warm environment. Nor were there any differences in body fat between the controls transferred to the cold at mating (gen 5) and the Eskimos. But their grandoffspring, reared in the cold (gen 7) had less absolute fat than the Eskimos. There was no difference, however, in percent body fat (P = Ø.16). At

generation 7 mice of either class in the cold were fattier than those in the warm. Although some of the difference in weight between the controls and Eskimos in the cold was due to fat, the lean weight of Eskimos was still greater than that of controls.

4.3.2 Generation 9

At generation 9 some of the control pairs, like those of generation 5, were transferred to the cold at mating and bred for two further generations.

4.3.2.1 Reproductive performance

The reproductive performance of controls transferred to the cold at generation 9 and of those of subsequent generations 1Ø and 11 was assessed as before (4.2.1) and compared with the reproductive performance of generations 9, 1Ø and 11 Eskimo mice. The results are given in Tables 4.8-1Ø.

There were more barren pairs among the controls than among the Eskimos; the proportion was about the same as for generation 5 transferred controls and smaller than that of generation 1 Eskimo (Table 4.8).

Again, one of the most notable effects of transferring the controls to the cold was the lengthening of the interval between mating and the birth of the first litter. This meant tat the controls produced fewer litters (Table 4.8) than the Eskimos and hence fewer young (Table 4.9). But unlike the earlier transfer pre-weaning mortality rates were much higher than those of the Eskimos except for generation 11 (Table 4.9). In this generation the Eskimos had an unaccountable increase in mortality rate. The high mortality rate may have been due to a greater percentage of smaller litters produced by generation 11 Eskimo.

Unlike all other classes in the cold the percent

loss of whole litters in generation 9 was only a small part of pre-weaning mortality for Eskimos and controls (Table 4.1Ø).

The young of the Eskimo mice were much heavier at 3 weeks than the young of control mice in both generations in which they were weighed (Fig. 4.5).

In summary, the Eskimos were superior to the controls transferred to the cold in nearly every aspect of reproductive performance measured.

Introductions to the cold.

The transfer of controls to the cold at generation 5 and 9 repeats initial transfer of mice from the warm to the cold laboratory at generation 1. It is interesting to see whether the same effects on reproductive performance were observed for all three transfers.

The long delays between mating and birth of the first litter were similar for all three transfers (Fig 4.3). The later transfers experienced somewhat longer delays than the first transfer. By the second and third generations in the cold, however, the delay was much shorter for the transferred controls than it had been for the equivalent generations of Eskimos. The pattern of losses of whole litters was the same for each transfer with a peak loss in the second generation in the cold (Fig. 4.2). The levels of mortality observed in the generation 5 transfer were lower than those observed in both the other transfers but mortality rates in the generation 9 transfer were little different from those of the initial transfer. The total preweaning mortality of generation 9 was much higher than indicated by the low % loss of whole litters (see above). Weaning weight of young increased over the generations in all three transfers (Fig. 4.5). However the young were heavier in both generation 5 and generation 9 transfers than in the initial transfer.

4.3.2.2 Body measurements and organ weights

The results of this transfer were similar to those of the transfer at generation 5 (Figs. 4.7-12). The pattern of body weight changes over the first three generations in the cold was the same for controls transferred at generation 9 as for those transferred at generation 5. Body length was more depressed in the latter transfer. By generation 9 the Eskimos were much bigger and heavier than they were at generation 5. In contrast the controls had changed little (Figs. 4.7-8). Hence the difference between controls and Eskimos in the later transfer was much greater than the difference observed in the generation 5 transfer.

The pattern of change in body size was different in the initial transfer from that of the later ones. At the initial transfer body size was the same as controls in the warm in the first generation and increased slightly thereafter. In the later transfers, the mice were initially slightly bigger than controls remaining in the warm but they were smaller in the second and third generations in the cold.

Tail length and consequently tail/body length ratio was similarly affected by the cold environment in all three transfers (Figs. 4.9-1Ø). The first generation in the cold had longer tails than later generations since the mice had been reared to mating age in the warm environment (see 4.3.1.2). The second and third generations in each transfer had tails which were about 75% of their body length.

Kidney weights increased relative to body weight on transfer to the cold environment in all three transfers and thereafter remained fairly stable (Fig. 4.12). Relative kidney weights of generation 9 transfers, however, changed fairly eratically. Although higher than control levels generation 9 transfers had slightly smaller

kidneys than those of the Eskimos. The kidneys of their offspring (generation 1Ø) were much heavier than those of the previous generation and slightly heavier than generation 1Ø Eskimos.

There was no difference in adrenal weights at any generation.

4.3.2.3 Body composition

Findings on the body composition of generation 11 control and Eskimo adult males in the cold are given in Table 4.16.

The relative fat content of the two groups was very similar. Hence the superior weight of the Eskimos was not due to a greater amount of fat but to a difference in total growth.

Similarly the relative fat content of the transferred controls in generation 7 was not different from that of the Eskimos.

4.4 Discussion

There was a marked difference between the two stocks in the changes observed over the generations. The controls, both adults and weaned young, increased slightly in weight, but did not change in reproductive performance. In contrast, the Eskimos became much larger; and they improved in nearly all aspects of reproductive performance. The temporal pattern of change was similar for most characteristics: the population changed slowly at first, but after generation 4 the rate of change accelerated.

One of the disadvantages of the method used in these experiments is that each domestication is a single operation; and each captive colony, in cold or warm, is a single population. There is therefore much scope for founder effects. For valid generalizations on the effects either of domestication or of cold adaptation, several such experiments are needed. But the only similar experiment is that of Barnett et al. (1975), on mice from a Scottish population, and hence likely to be genetically different from those I used.

There were nonetheless some similarities in my findings and those of the Scottish experiments.

(i) Both populations of wild mice increased in weight over the generations although the increase in size of the Eskimo mice of the present study was greater.

(ii) Initial high infant mortality followed by gradual improvement was observed in both populations.

(iii) In both populations total number of young born per pair increased over the generations.

More important are the differences from the findings of Barnett et al. (1975).

(i) The young of the Scottish wild mice grew faster than controls soon after transfer to the cold and showed no further change over the generations; in contrast the

growth of the young of Eskimo mice was initially depressed and showed gradual improvement over the generations.

(ii) There was a high proportion of barren pairs in every generation of Scottish mice whereas there was a decline in the number of barren pairs over the generations in the present study.

(iii) There was a progressive increase in the litter size of the Scottish mice but the litter size of Eskimo mice remained stable over the generations.

(iv) Although both populations showed an increase in total number of young born per pair the causes of the increase were different; increasing litter size over generations was the main cause of increase in the Scottish mice whereas gradual reduction in delays to breeding was the cause of increase in the Eskimo mice.

Increase in body size appears to be an adaptive response to living in a cold environment. A smaller surface to volume ratio would reduce heat loss. This is the theory behind Bergman's rule concerning the latitudinal cline of weights within a species. The generality of this rule does not always hold in nature (Scholander, 1955). Berry et al. (1978), however, found mice from cold climates to be heavier than those from warm ones. Certainly the results of the present study support the assumption that increase in body weight is an adaptive response to the cold. In nature, such a response might be modified by shortage of food and other factors. In fact, Iverson and Turner (1974) postulated that the low weight of animals in autumn was an adaptive response to reduce food needs in winter.

Delays in the onset of breeding, high infant mortality and reduced growth seem to be common responses to cold (Narzian and Piacsek, 1977; Barnett, 1973). These were features of the early generations of Eskimo mice. Populations vary, however, in the type and extent of change over many generations in a cold environment. The

Eskimo mice improved in all aspects of reproductive performance except litter size. Inbred laboratory strains breeding at -3 C remained unchanged except for a decline in nestling mortality (Barnett, 1965). The litter size of hybrid laboratory strains bred at the same temperature increased over the generations, but so did the litter sizes of controls remaining in the warm.

It is interesting to look at characteristics which changed over the generations but it is of equal value to consider some of the characteristics which did not change.

Relative kidney weights were always higher in the cold but there was no change with generations in either environment. When controls were transferred to the cold in generations 5 and 9 their relative kidney weights were similar to those of the Eskimos. Eskimos transferred to the warm similarly had relative kidney weights nearly identical to those of the controls. The kidney is a very plastic organ. It can change rapidly in size with need during an individual's lifetime; in pregnancy and lactation (Matthews, 1977; Barnett, 1965,1973), and also when one kidney is removed. There may have been changes in the efficiency of the kidney but the ontogenetic increase in size seemed to be sufficient to cope with the extra demands of higher metabolism in the cold.

The ratio of tail length to body length remained constant throughout the generations in both temperatures although the ratios of the control mice tended to oscillate from generation to generation. There was no proportional increases in tail length similar to that observed with the Scottish wild mice (Barnett et al., 1975).

The litter size of the Eskimo mice did not increase over the generations although larger litters had a greater chance of survival (possibly related to the higher nest temperatures in those litters, see 3.2.2). Also increase in litter size commonly accompanies increase in body size

of domestic mice (Falconer, 1955). And finally, strains of genetically heterogenous mice, including wild mice, increased in litter size at birth over many generations at -3°C (Barnett, 1973).

Although there was an immediate effect of captivity on the size of the wild mice, the body proportions and relative kidney weights of the trapped mice were were similar to those of the controls kept at 23°C. Since the trapped mice were caught in early autumn they had probably spent most of their lives in warm temperatures. Hence their similarity to the controls in bodily proportions could be explained by the similarity of temperatures.

The aim of the transfer experiments was to see to what extent the two populations had diverged by comparing them in the same environment.

There was little difference between the Eskimos and controls in the warm environment at generations 5, 6 and 7. The growth rate of the Eskimo young was, however, higher and the tails of the Eskimos were proportionally longer than the controls. The tail is one of the principal areas for heat dissipation. Hence, the longer tails may have been an ontogenetic response to a greater need for heat dissipation in the Eskimos perhaps due to a higher metabolic rate. There was, however, no marked superiority of the transferred Eskimo mice over the controls as observed by Barnett with Scottish wild mice. A more relevant comparison would be between generation 1Ø mice transferred to the warm and the Scottish transfers but Eskimos were not transferred to the warm at generation 1Ø. Looking at the total pattern of change in the Eskimo mice it seems that little change had occurred by generation 5. The rate of change was greater after generation 5.

At generation 5 in the cold, however, the Eskimos were superior in some respects to the controls newly

transferred to the cold. The nestling mortality of the transferred controls was, however, no different from that of generation 5 Eskimos; the high infant mortality rates observed in the first few generations of Eskimo mice were not repeated. Breeding for some generations in a laboratory conditions evidently led to a change among the controls that enabled them to cope better with the cold environment.

By generation 9 the Eskimos were superior to the newly transferred controls of generation 9 in all aspects of reproductive performance including infant mortality. But, like the controls of generation 5, they adjusted more quickly to the cold than did the first few generations of Eskimos. The pre-adaptation to the laboratory by the controls again may have given them an advantage in cold adaptation.

The Eskimos of generations 9, 1Ø, and 11 were much bigger than the controls in the warm or the cold. It was unfortunate that transfer of Eskimos to the warm at generation 9 was not carried out. It would have been interesting to see if the Eskimos showed a similar superiority to controls in the warm.

Litter size and the growth of the young.

One factor that influences body weight from birth or before is litter size. The effect of litter size on growth of individual young is discussed here because of its general interest and implications in the study of maternal effects (Falconer, 1965).

The simplest model of the effect of litter size is that of a simple negative linear regression of individual weight on litter size; and this is often held to represent the facts (Turner et al., 1976; Nelson and Robison 1976; Drickamer, 1976; Nagai, 1971; Widdowson, 1968; Dobbing and Widdowson, 1965; Milkovic et al., 1976,1977; Fuchs, 1982; Crozier and Enzman, 1935). The decrease in individual

weight with increasing litter size is assumed to be due to the limited ability of the mother to adjust milk production to litter size. The effect of increasing litter size is thought to be that of a decrease in nutrition for the individual. This undernutrition is thought to be sufficiently important to reduce body growth permanently by reducing the rate of cell division and final number of cells per organ (Widdowson, 1968).

The observations of the present study do not conform with this model. The Eskimo mothers, in particular, were able to increase milk production with increasing litter sizes. The controls adjusted milk production up to a certain litter size whereafter individual weights declined with further increase in litter size. Similar observations were made by Barnett et al. (1975). No effect of litter size on weaning weights was observed with one group of wild mice and in another group an effect of litter size was apparent only at low sizes; the wild mice did not appear to have an upper limit.

Some studies on laboratory mice have also shown that a simple linear regression is not the best model. Shibata (1965) found the correlation coefficients between litter size and mean body weight at weaning to be high and negative; but multiple range tests showed that litter sizes in the middle range (5-9) were heavier than litters of 2-4 or 10+. Also the weaning weight of laboratory mice kept at -3^{o} C declines with increasing litter size only above a certain size; the upper limit varying among strains (Barnett and Neil, 1972). Rath and Thenen (1979) found that litter size did not affect the mean milk intake of 10-day mouse pups. For a litter of up to 10 pups milk production of the mother was adequate to maintain the mean intake of the pups between 0.9 and 1.0 ml.

Many of the investigations into the effects of litter size on growth of young involve fostering young between mothers at birth to produce standard litter sizes.

Mothers then often rear abnormally small or large litters, unrelated to the size of their natural litter. When litters are unmanipulated, as in the present study, the sizes of the litters are more likely to be within the physiological capabilities of the mother. There is undoubtedly natural selection operating on litter size (Lack, 1948).

The difference between wild and domestic mice in the effect of litter size may be more apparent than real. Similar manipulation of wild mice litters might also show a negative linear regression of weight of young on litter size.

In the present study the effect of litter size on the growth of the young was minimal. Over the range of natural litter sizes the wild females were able to adequately adjust milk production so that growth was similar in all sized litters.

5. EFFECTS OF MATERNAL ENVIRONMENT

The aim of the following experiments was to distinguish between maternal and genetical effects and to assess their contribution to differences between the warm-adapted and cold-adapted mice.

5.1 Experimental design

The differences between the warm-adapted (control) and cold-adapted (Eskimo) mice observed in the same temperature (4.3) were assumed to be due to (i) genetic change (from selection by cold and genetic drift) or (ii) cumulative maternal effects (see Fig. 1).

One way of partly distinguishing maternal and genetic components is to cross-foster litters at birth. In this way postnatal maternal effects can be separated from the combined effects of the prenatal maternal environment and the genotype. The cross-fostering procedures are described in Section 3.2.1. Cross-fostering was carried out in both environments among parents from generation 6. Litters were fostered between residents and transfers (which had been reared from birth in the new environment) and within classes as controls. The results of the measurements made on the fostered mice are presented in section 5.2.

Another method of measuring the relative contributions of maternal environment and genetic components is reciprocal mating. This provides a control for combined pre- and post-natal maternal effects. At generation 1Ø the Eskimos were mated reciprocally with controls; the latter were the offspring of generation 9 controls which had been transferred to the cold at mating. Two classes of hybrid were produced, one with Eskimo mothers and one with control mothers. The assumption was that these two classes were genetically identical and that differences between them could therefore be attributed to different maternal environments. It was assumed that sex linkage was negligible for the characters measured. Details of the procedure are given in Section 3.2.2.

5.2 Cross fostered mice

5.2.1 Growth

The cross fostered mice were weighed individually at birth, 10 days and at 21 days. Between-litter variance was always much higher than within-litter variance (3.1.5). Mean individual weight per litter (males and females combined) was therefore used as the replicate. Males only were weighed at 10 weeks and 16 weeks, and here each animal was used as the replicate. Males from the mated pairs were also weighed at the end of the reproductive period, at about 30 weeks of age.

Fostered litters were reduced to 5 at birth to control for possible effects of litter size on growth. It was still possible that litter size in utero affected later growth; but in fact no evidence was found of correlation at any age between weight and litter size at birth, for any class. Nor, in most classes, was there any evidence of correlation between weight and parity of true mother.

The pattern of growth of the fostered mice in both temperatures is illustrated in Fig. 5.1; the first three weeks are shown in detail in Fig. 5.2.

Eskimo mice grew faster than controls in both temperatures, although in the warm their weight at 30 weeks was similar to that of the controls. There was no growth-enhancing effect of Eskimo foster parentage on control mice in either temperature, but there was a growth-retarding effect of control foster parentage on Eskimo mice. This effect was especially marked in the cold environment. There was evidence of compensatory growth among the Eskimos with control foster parents after the end of the lactation period (Fig. 5.2), but the Eskimos with control foster parents did not reach the heavier weights of the within-class fostered Eskimos at 30 weeks.

The effects of temperature, foster parents, and true

parents (genotype + prenatal environment) are summarized in three-way analyses of variance (Tables 5.1-5). True parentage had the predominant effect on birth weight ($P < 0.001$): Eskimos were heavier at birth than controls. Temperature had a small effect ($P = 0.042$): Eskimos in the cold were heavier at birth than those in the warm. There could, of course, be no effect of foster parent type on birth weight.

At 10 days true parentage and foster parentage type had an approximately equal influence ($P < 0.001$), but there was also an interaction. Although control foster parents retarded the growth of their Eskimo foster young, there was no foster parent effect on control mice. Ambient temperature exerted a small influence ($P = 0.034$): 10-day mice in the warm were slightly heavier than those in the cold.

By 21 days, there was still an effect of foster parentage ($P = 0.001$) but it was not as great as that of true parentage ($P < 0.001$); and there was again an interaction. The effect of temperature on 21-day weight was greater than at previous ages ($P < 0.001$): mice in the warm were much heavier than those in the cold.

At 10 and 16 weeks only true parentage had an effect on weight: Eskimos were again much heavier than controls. There was no evidence of an effect of foster parent or of temperature.

5.2.2 Reproductive performance

The reproductive performance of the fostered mice is presented in Tables 5.6-12 and Fig. 5.2.

In the warm environment there was little difference between the four fostered classes in most aspects of reproductive performance. The only difference due to foster parentage was in litter sizes. The litter sizes of Eskimo pairs which had control foster parents were smaller than those of the other three classes (Table 5.8). That

is, control foster parentage affected the size of the litters produced by their Eskimo foster young. Control mice were unaffected by foster parentage. There was a consistent effect of true parentage on the 3-week weights of the young; Eskimos reared heavier young than controls, regardless of foster parentage (Fig. 5.3).

In the cold environment there were more differences between the fostered classes. The total number of The total number of litters born per pair and consequently the total number of young born were influenced mainly by true parentage (Table 5.6). Eskimo mice tended to produce more litters in a given period than did controls, regardless of foster parentage. This was due to a shorter interval between mating and birth of first litter (Table 5.9).

There was no difference in pre-weaning mortality between any classes (Tables 5.6 and 5.7).

There was a foster parent effect on litter size at birth; control mice with Eskimo foster parents had larger litters than control mice with control foster parents (Table 5.8). There was an indication of a similar effect of foster parentage on Eskimo mice (but P = Ø.111). A two-way analysis of variance showed an effect of fostering on the litter sizes of foster young, with no interaction, that is, the foster parent effect was in the same direction for both classes of foster young (Table 5.11).

There was also a foster parent effect on weaning weight (21d) of the young of fostered mice (Fig 5.3): young of Eskimo mice which had had control foster parents weighed less than young of Eskimos which had Eskimo foster parents. A two-way analysis of variance of three-week weights by true parentage and foster parentage showed no good evidence of an effect of foster parent (P = Ø.Ø89); but there was an interaction between true parentage and foster parentage (Table 5.1Ø). The effect of Eskimo foster parentage was not the same for both types. It had no effect on control mice; yet Eskimo mice with Eskimo

foster parents had heavier litters than those with control foster parents. This pattern repeats that of the fostered mice themselves (5.2.1). The depressing effect of control foster parents on their Eskimo foster young was carried on in the offspring of the fostered mice.

5.2.3 Body measurements and organ weights

Adult males were weighed and measured at two ages: 16 weeks and 3Ø weeks or older (Tables 5.13-14).

A three way ANOVA was carried out for body weight and length and tail length of males aged 16 weeks (Table 5.15).

There were strong effects of true parent, temperature and foster parent on all three measures. The effects on body weight and length were similar. True parent had the largest effect. Eskimos were heavier and longer than controls. There was also an interaction between true parent and temperature. Control mice weighed less and were shorter in the cold than the warm but Eskimo mice were the same in both temperatures. Temperature had by far the greatest effect on tail length: tails were shorter in the cold (see 4.3.1.3). True parentage also had an effect, Eskimos had the longer tails. Foster parentage too, had an effect; mice with Eskimo foster parents had longer tails than those with control foster parents. foster-parentage also had some effect.

We now look at the findings on the body size of the 3Ø-week old males. In the warm there was no difference between any class in body weight or length, except that Eskimo mice were slightly longer than control mice in the control foster-parent class. Eskimo mice, whatever their foster-parents, had longer tails than control mice. There was, however, little difference between classes in the ratio of tail to body length. Hence in the warm the slight foster parentage effects evident among the 16 week old mice had disappeared by the time the mice were 3Ø

weeks.

In the cold, in contrast, there was an even more marked effect of control foster-parentage on Eskimo mice than there was at 16 weeks. Eskimo mice with control foster parents were lighter and shorter than Eskimos with Eskimo foster parents. There was not, however, an opposite effect of Eskimo foster parentage. Controls with Eskimo foster parents were no different from those with control foster parents. There were no differences between any class in tail length or tail/body length ratio.

Table 5.16 gives the results of three-way analyses of variance. The results for body weight and length were again similar. True parentage had the predominant effect, far outweighing the effects of foster parentage or temperature.

As with the 16-week males temperature had the greatest effect on tail length but true parentage and fostering still had some effect.

The findings on the kidney and adrenal weights of mice aged 16 and 30 weeks are presented in Tables 5.17-18. In the warm the relative kidney weights of the 16-week old males from the between-class fostered groups were higher than those of both the within-class fostered groups. At 30 weeks of age, however, there was no difference between any classes in kidney weight except for the Eskimos with Eskimo fosterparents. These mice had heavier kidneys both absolutely and relatively than any of the other classes. This is the unusual group described in 4.3.1.3.

In the cold there were no differences between classes in relative kidney weights at either age.

There was no differences in adrenal weights between classes in the warm at either age.

In the cold the controls had heavier adrenals than Eskimos, whatever their foster-parentage, at 16-weeks of age and at 30-weeks the controls with control foster

parents had heavier adrenals than any of the other three classes.

5.2.4 Body composition

Body composition was analysed only of males aged 30 weeks or older (Table 5.19).

In the warm there was no difference between any of the classes in fat content. In the cold, however, there was further evidence of the effect of control foster parentage on Eskimo mice. Eskimo mice with control foster parents had less fat both absolutely and relatively than those with Eskimo foster parents. There was no difference in lean weight between the two classes; hence the difference in total body weight due to foster parentage (Table 5.11) was due to differences in fat content.

5.3 Reciprocal matings

5.3.1 Reciprocally mated pairs

Information on the four classes of parents (male parent first: control x control; control x Eskimo; Eskimo x control; Eskimo x Eskimo) is presented here as background information.

5.3.1.1 Reproductive performance

The present work is concerned especially with maternal effects. But examination of the reproductive performance of the reciprocally mated pairs showed that a simple interpretation in terms of a consistent superiority of Eskimo mothers is not valid (Tables 5.20-23). It was necessary to take into account paternal as well as maternal influences. Thus reproductive performance was examined as a 2 by 2 situation; 2 types of males and 2 types of females. Two-way analyses of variance were performed on total number of young born per pair and on percentage of young reared to 3 weeks to investigate the effects of different male/female parent types (Table 5.24). These analyses disregard the possible influence of different types of young on these reproductive measures. The type of female had a substantial effect on the total number of young born per pair. There was also an interaction between male and female type. Eskimo females had more young than control females, but the performance of control females also differed according to mate type. The effect of having an Eskimo mate was to improve the performance of the control females in number of young born, both by reducing the interval between mating and the birth of the first litter (Table 5.23) and by increasing litter size (Table 5.22). There was, however, no analogous effect on Eskimo females of having a control mate. In fact, Eskimo females with control mates had a shorter delay between mating and the birth of the first litter than Eskimo females with Eskimo mates.

The percentage of young reared is influenced both by whole litter losses and by part litter losses. The two-way analysis of variance (Table 5.24) demonstrates a considerable effect of the male. Pairs with Eskimo males reared a higher proportion of young than those with control males. The effect of the female was negligible. Mortality rates are, however, also influenced by the survival abilities of the young. These were likely to differ among the three groups of young: control, Eskimo and hybrid. The reciprocally mated pairs, Eskimo x control and control x Eskimo, both reared hybrid young which presumably would have similar survival potentials. The pairs with control males had a higher mortality rate than those with Eskimo males (but P = Ø.Ø59) (Table 5.21).

At mating, Eskimo females were heavier than control females (Table 5.25) and therefore a maternal effect on the weight of young was expected. The growth rate of the young from birth to three weeks depended on the type of the mother; the type of father had no effect (Fig 5.5). The Eskimo females produced heavier young at birth and reared heavier young than the control females. The type of mother also affected the correlation of 3-week weight with litter size and with parity (Table 5.26). There was a negative correlation of litter size and three week weight and a positive correlation of parity with 3 week weight for both groups of mice with Eskimo mothers, but there were no such correlations for the offspring of pairs with control females. There was no correlation for any class between litter size and parity. When the pairs were grouped according to type of female, the differences between the two groups according to type of mother was clearly seen.

5.3.1.2 Body measurements and organ weights

The findings on body measurements and organ weights are presented in Tables 5.27-28.

The measurements were made at the end of the reproductive period when the males were killed. Differences between mouse types are described in 4.3.2.3 and are not repeated here. There was no difference in any measure within mouse type due to different mate type, except that Eskimos with Eskimo mates were slightly longer than those with control mates. Since the former class was kept for longer after the end of the breeding period for milking experiments the difference in age could explain this result.

5.3.2 Growth of hybrids

The offspring of the reciprocal matings and the offspring of generation 1Ø controls and Eskimos in the cold were weighed individually at birth, 1Ø days and 21 days. Between-litter variance was always much higher than within-litter variance (3.1.5), so mean individual weights per litter (males and females combined) were used as the replicates. Males were also weighed at 6, 1Ø, and 16 weeks, and again mean individual weights per litter were used as the replicates. At about 3Ø weeks males from the mated pairs were weighed; in this case individual weights were used for statistical analysis. The results of the weighings are illustrated in Fig. 5.4; the first few weeks are enlarged in Fig 5.5. The number of litters weighed for each class are given below.

age	controls	Eskimos	Esk x con	con x Esk
Ø	26	36	29	33
1Ø	28	46	43	31
21	24	47	38	31

The growth of the two hybrid groups is of primary interest since any difference would be due to differing maternal environments. The hybrid mice with Eskimo mothers were heavier at birth than the hybrids with

control mothers. This difference between the two groups of hybrids was maintained until at least 16 weeks. The weights of the 3Ø week old hybrid males were, however, similar.

By comparing the hybrids with the Eskimos and controls the effects of genotype on growth can be seen. During the period from birth to 3 weeks the type of mother had the predominant effect on weight: Eskimo mothers reared heavier young. Mice with the same type of mother weighed the same regardless of genotype. After 3 weeks the mice with the same type of mother began to diverge according to genotype: by 6 weeks hybrids with Eskimo mothers weighed less than Eskimos and hybrids with control mothers weighed more than controls. Between 3 and 6 weeks the hybrids with control mothers grew relatively faster than the other classes (Fig. 5.6). After 6 weeks, however, the hybrids with control mothers did not differ from controls. The 3Ø week old hybrid males weighed the same as the controls despite differences in genotype. At 3Ø weeks the Eskimos were much heavier than all three classes.

5.3.3 Reproductive performance of hybrids

At mating, at about 6Ø days, females were weighed (Table 5.25). Eskimos were, as usual, heavier than controls, but there were no differences between the two hybrid groups. There were differences in the weights of males weighed at a similar age: hybrid males with Eskimo mothers were heavier than those with control mothers (5.3.2).

The findings on the reproductive performance of the offspring of the reciprocal matings (that is, of the hybrids) and that of generation 11 controls and Eskimos, are presented in Tables 5.29-32. The number of litters weighed in each class is given below.

age	controls	Eskimos	hybrids with control mother	hybrids with Eskimo mother
Ø	14	9	18	27
1Ø	24	25	19	36
21	24	21	18	37

The two hybrid classes differed in total number of young produced (Table 5.29). Those with Eskimo mothers produced a mean of about 4Ø young, whereas those with control mothers produced only half as many. This was partly because they produced more litters, as a result of a shorter delay before the birth of the first litter (Table 5.32); but a difference in litter size also contributed (Table 5.31).

The hybrids resembled the stock from which their mothers originated. That is, pairs reared by Eskimo mothers had more litters and produced a greater number of young than pairs reared by control mothers. Eskimos had smaller litter sizes than hybrids with Eskimo mothers; but, when litters which did not survive were exluded, there was no longer a difference in litter size between the two classes.

The hybrids with Eskimo mothers were also more successful at rearing their litters than were those with control mothers (Table 5.29-3Ø). As a result of the lower mortality and greater production the hybrids with Eskimo mothers weaned three times as many young as those with control mothers (Table 5.29). Hybrids with control mothers resembled generation 11 controls in most aspects of pre-weaning mortality. But the hybrids with Eskimo mothers had lower mortality rates than the Eskimos. Generation 11 Eskimo were, however, different from previous Eskimo generations; their mortality rates were unaccountably much higher (4.3.2.1). When the mortality rates of the hybrids were compared to those of the

previous Eskimo generation there was a close resemblance in terms of pre weaning mortality rates. It is more likely then that the differences between the two groups were due to the generation 11 Eskimo being atypical rather than the hybrids with Eskimo mothers demonstrating a form of hybrid vigour.

Although the two classes of hybrids differed markedly in most aspects of reproductive performance, there was no difference between them in the weight of their litters, at birth, 1Ø days or 21 days (Fig. 5.7). The two groups of hybrids weaned young with a mean weight midway between the two parental means. During their first 1Ø days the young with Eskimo grandmothers tended to grow faster than mice with control grandmothers (Fig. 5.8); but between 1Ø and 21 days the reverse was true. So there was a small grandmaternal effect evident only during the lactation period.

There were no correlations of 3-week weight of offspring with litter size or with parity (except that the 3-week weights of controls were correlated with parity) (Table 5.33).

5.3.4 Body measurements and organ weights of hybrids

Adult males were again measured at two ages: 16 weeks and 3Ø weeks or older (Tables 5.33-34).

The main concern of the present chapter is the differences between the two hybrid classes. Differences between mouse types are described in 4.3.2.3. At 16 weeks there was a large difference between hybrid types in all measures. The hybrids with Eskimo mothers were heavier and longer, and had longer tails and a higher tail to body length ratio, than the hybrids with control mothers. There were also differences due to genotype. The Eskimo x Eskimo were heavier and longer than control x Eskimo; and Eskimo x control were longer with longer tails but not heavier than control x control. These differences,

however, were smaller than the maternal effects.

At the age of 3Ø weeks or older the differences between the hybrids were smaller. Although the hybrids with Eskimo mothers were longer than those with control mothers they were not heavier. The Eskimo x Eskimo males, however, were much heavier and longer than males of the other three classes.

There was no difference in relative kidney weights between the classes at 16 weeks, but at 3Ø weeks the Eskimo x Eskimo had lighter kidneys than either group of hybrids. There were no differences in adrenal weights at either age (Tables 5.35-36).

5.3.5 Body composition of hybrids

Body composition was analysed for males aged 3Ø weeks and older only (Table 5.37).

The results did not show any differences between classes in fat and water content. The numbers were, however, small and variances were high. Calculation of fat-free body weight showed that the Eskimos had a heavier lean weight than the other classes; hence the differences in total body weight were not due to differences in fat content.

5.4 Discussion

I now examine the main findings concerning maternal effects, separately for each experiment, and draw tentative conclusions. Suggestions are also made for improved experimental design.

Cross Fostering. With the benefit of hindsight it seems that one of the main errors of the fostering experiment was that it was performed too early in the adaptation of the Eskimos to cold. When the Eskimos were transferred to the warm at generation 5 they were little different from the controls. Therefore little would be expected in the way of maternal effects. In the cold environment, however, differences between the Eskimos and the controls were more marked. Similarly maternal effects were greater in the cold.

The maternal effects were asymetrical. There was no effect of Eskimo foster parentage on the growth of controls in either temperature. But the growth of Eskimo young was retarded when they were reared by control foster parents. This resembles the finding of Al-Murrani and Roberts (1978). The postnatal maternal effects they observed were largely due to the inadequacy of small strain mothers to rear large strain pups.

The retardation of growth due to control foster parentage affected the weight of adult Eskimo, although there was some compensatory growth. Most of the adult weight difference between Eskimos with control foster parents and those with Eskimo foster parents was due to fat: their lean weights were similar. Perhaps early postnatal growth retardation affects the laying down of fat cells (Widdowson, 1968).

Of primary interest is that the retarding effect of control foster parents on Eskimos was carried on to the

next generation. The young of Eskimos who had been reared by control foster parents were smaller at three weeks than the young of Eskimos who had been reared by Eskimo foster parents. Suprisingly, a 'grandmaternal' effect was also observed with control mice. Although there was no enhancing effect of Eskimo foster parentage on the growth of controls the size of their subsequent litters was affected. Early Eskimo rearing led to production of larger litters.

Reciprocal mating. The experiment on reciprocal mating was reduced to a basic design containing only 4 cells owing to constraints on time. More information would have been gained by (i) transferrring a group of Eskimos to the warm at generation 9 and duplicating the experiment there, and (ii) cross fostering at birth between the two sets of hybrids, Eskimos and controls. The latter experiment would have allowed separation of maternal effects into preand postnatal components.

Since one of the main objectives of the experiment was to examine the effect of the mother on growth more female weights should have been recorded; in particular, unmated females at regular intervals until 3Ø weeks of age and breeding females at the birth of each litter. This would have made possible a more detailed investigation into the relationships between the weight of the mother and that of her young. But, despite the limitations of the experimental design some interesting findings emerged.

Birth weight and growth to the age of 3 weeks were determined by the type of mother irrespective of genotype. Hybrids with Eskimo mothers grew at the same rate as Eskimos; and hybrids with control mothers grew at the same rate as controls. After 3 weeks the effect of genotype became evident. Hybrids with control mothers grew faster than controls and hybrids with Eskimo mothers grew slower than Eskimos. But maternal effects on male weights were

still large up to at least 16 weeks of age. There was no difference, however, between the two hybrid groups in female weights at about 9 weeks of age. The females weighed at this age were only a small sub sample but they were chosen randomly and should have been representative of their class. It is interesting to speculate why there was a persistent maternal effect on male but not female weights. Male and female growth curves diverge after weaning: females grow more slowly than males. Perhaps the growth of females after weaning was too slow and the within class variation too great for maternal effects to be evident. This seems an unlikely explanation. It may be that there is a genuine sex difference in response to maternal effects. This would require further investigation.

The weights of males aged 3Ø weeks or older pose a problem. The two hybrid groups and the controls were the same weight. It is not surprising that the two hybrid groups should converge at this age; maternal effects commonly diminish with age (see Introduction); but it is surprising that the hybrids should be the same weight as the controls. One would expect them to weigh midway between the two parent groups.

Unfortunately, only 1Ø males were weighed in each class, and these were from the mated pairs. So numbers were small and the sample differed from the sample of males weighed at earlier ages. Hence these findings only indicate an area to be investigated.

The demonstration of maternal effects in reproductive performance is even more striking than the demonstration of their involvement in growth. Despite the fact that both groups of hybrid females weighed the same at mating the reproductive performance of the hybrids with Eskimo mothers was superior to that of the other hybrids in nearly every respect. Delays to breeding were shorter, litters were larger and a much higher percentage of young

was reared. The pre- and postnatal environment of the Eskimo mother affected the reproductive performance of her hybrid offspring in a way which was unrelated to body size.

Yet there was no marked 'grandmaternal' effect. The 3-week weight of the offspring of both hybrid groups was midway between the two parental groups. There was, however, an indication that some 'grandmaternal' effect was operating, since the young of the hybrids with Eskimo mothers grew faster up to 10 days of age than young of the hybrids with control mothers.

Perhaps the 'grandmaternal' effect was modified by the larger litter sizes of the hybrids with Eskimo mothers. But there was no negative correlation between litter size and weight of the young: the young from larger litters were as heavy as those from the smaller litters. Hence it is unlikely that the difference in in litter sizes between the two classes had any bearing on differences in growth of the young.

General Conclusions. Both experiments demonstrated clearly maternal influences on growth. This finding is matched by much evidence in the literature; but authors differ widely on the extent of maternal influence (see Introduction).

The maternal effects differed in direction and extent between the two present experiments. Eskimo foster parentage had no enhancing effect; but in the reciprocal mating experiment the combined pre and postnatal effects of Eskimo mothering enhanced the growth of the hybrids. It is unlikely that the enhancing effect of the Eskimo mothers was due entirely to the prenatal component. Probably the maternal efficiency of the Eskimos of generation 10 was superior to that of generation 6. The maternal effects clearly persisted beyond weaning age: in both experiments they influenced adult body weight and

later reproduction to varying extents.

Of crucial importance is whether the maternal effects are carried over to at least the next generation. The results from the two experiments are equivocal. Maternal effects continued to influence weight up to and beyond the breeding age of the offspring and therefore could be expected to influence the next generation. The growth-retarding effects of control mothers rearing Eskimo young were carried on to the next generation. This observation resembles the effect of malnourished grandmothers on learning deficits in rats (Bresler et al., 1975) and also the long lasting effects of malnourishment in World War II on women of that generation and the next.

The growth-enhancing effect of Eskimo mothers observed in the reciprocal mating experiment did not, however, carry over to the next generation. Whether there is a difference in the persistence of maternal effects depending on whether they are detrimental or beneficial cannot be answered by these experiments.

It was unfortunate that the offspring of the fostered and hybrid mice were not studied after 3 weeks of age. Records of their further growth and reproductive performance and perhaps the growth of their offspring might have shown further persistence of maternal effects.

These experiments, although they show that maternal effects are operating, can give no evidence to support the theory that the accumulation of maternal effects over the generations can help the population in adaption to a new environment. To test this theory a design involving cross fostering experiments over several generations would be required.

6. THE MATERNAL ENVIRONMENT - MILK SUPPLY

As outlined in the introduction (1.1) the aim of the following experiments was to examine aspects of the postnatal maternal environment of cold-adapted and warm-adapted mice.

6.1 Experimental Design

Variations in the postnatal growth of young attributable to maternal effects are likely to be due largely to differences in the quality and quantity of the milk. For this reason I examined the milk production of three classes of mice at generation 1Ø. The classes were: (a) controls in the warm; (b) controls whose parents had been transferred to the cold at mating (immigrants); (c) Eskimos in the cold. 1Ø day old mice from these 3 classes were also analysed for body composition. The experimental design would have been improved by studying the Eskimo mice also in the warm, but this was not possible in the time available.

6.2 Milk Composition

The results of the milk analyses are presented in Table 6.1 and Fig. 6.1. The values for cow's milk closely match those reported elsewhere (Spector,1956). The figures for mouse milk, as expected, varied more than those for cow's milk. The mouse milk was taken from different females at different times, whereas the cow's milk consisted of sub-samples of a single batch of raw milk from many cows. The values for the Eskimo milk resembled those reported elsewhere for laboratory mice at the same stage of lactation (Baverstock et al., 1976; Hanrahan and Eisen, 197Ø). Rath and Thenen (1979) report similar values for milk fat but values for total solids were lower. The values for total solids, fat and protein from the milk of control mice were much lower.

The specific gravity of the mouse milk from all classes was 1.Ø3 and was identical to that of cow's milk. The proportion of total solids was, however, higher than that of cow's milk. Fat and protein evidently increase together and so the specific gravity is unchanged. The Eskimo mice had a much larger proportion of solids in their milk than had either controls or immigrants: fat and protein levels were both higher. In particular the Eskimo mice had nearly twice as much milk fat as the controls. There was also an effect of temperature, evident in the differences between the two classes of control mice. Those in the cold (immigrants) had more fat but less protein than those in the warm. Total solids, however, were the same in the two groups.

In summary, the milk of the Eskimo mothers in the cold was richer than that of control mothers in either temperature. The milk of the immigrants did tend to have more fat and less protein than the milk of the controls; but the effect of temperature was small compared to that of the difference between classes.

6.3. 24-h Milk Intake of Infant Mice

For both pup body weight and milk intake between-litter variance was, as expected, higher than within-litter (Table 6.2). For statistical analysis, therefore, mean litter values were used, and each N was the number of litters.

The findings on milk intake are given in Table 6.3. Maternal weights, at the time of experiments, differed between the three classes: controls were lighter than both groups in the cold, and Eskimo mothers were heavier than immigrants. Although immigrant mothers were heavier than control mothers there was no difference in the 10-day weight of their pups. But maternal weights recorded were only of mothers which had reared litters to 10 days. The two groups may have differed because only the heavier females produced litters in the cold.

The weight of all females had been recorded at mating (Chapter 7). The immigrant females were grouped into (a) successful(n=6) and (b) unsuccessful, that is, barren or those whose litters died soon after birth (n=4). The mean weight at mating of the successful females was 12.25+0.36 and that of the unsuccessful was 10.5+0.45. Hence the successful females tended to be heavier ($P<0.05$); but the difference was small.

Eskimo pups at 10 days were heavier than controls or immigrants.

Pups of both classes in the cold environment drank more milk in 24 hours than those in the warm, and Eskimo pups drank more milk than immigrant pups; but milk intake was correlated with pup weight in all classes (Table 6.4) so milk intake was divided by body weight to give a proportional measure of milk intake (Table 6.3). The difference in milk intake due to temperature is clearly seen. Controls drank about 15% of their body weight in

milk daily, but the two groups in the cold drank about 23%. Although the Eskimos drank a greater proportion of milk than the immigrants, the difference was small.

Pup weight, milk intake and maternal weight were all intercorrellated (Table 6.4). The slopes of the regressions did not differ among classes. There was a large amount of variation in the maternal weights of the Eskimos so correlations between maternal weight and pup weight or milk intake were of low statistical significance.

A multiple regression equation was computed to explain pup weight as a function of class effects (mouse type and temperature), milk intake and maternal weight (Table 6.5).

In the form $y = a + bx + cz$

Where

y = pup wt

a = y intercept

b = slope of the regression of pup wt on milk intake

x = milk intake

c = slope of the regression of pup wt on maternal wt

z = maternal wt

Since the classes did not differ in the slopes of the regressions of milk intake and maternal weight on pup weight the same the same equation was applicable to all three classes, but with a different y intercept value for each class. Substituting the values from the multiple regression (Table 6.5) the equation becomes -

pup wt = a + 1.896(milk intake) + Ø.Ø63(maternal wt)

a = 2.3Ø for controls

= 1.22 for immigrants

= 1.Ø9 for Eskimos

62% of the variance in pup weight can be explained by this

model. The effect of removing components of the equation was tested (Table 6.6). Removal of the maternal weight component caused a mean change in the deviance of the model of 1.13 (P = Ø.Ø6). Without maternal weight the model accounted for 57% of the variance in pup weight. Removal of the milk intake component had a much larger effect. Mean change in the model deviance was 3.27 (P < Ø.Ø1) and the percentage of variance in pup weight explained by the model without the milk component was 47%. Removal of the class effect component resulted in a mean change in the model deviance of 1.83 (P < Ø.Ø5). The variance explained was again 47%.

Hence milk intake was the largest contributor to the variance in pup weight. Class effects, mainly temperature, were also important. The effect of maternal weight was smaller.

The relationship between pup weight and milk intake is further illustrated in Fig. 6.2. Again the effect of temperature on milk intake is demonstrated. Both groups in the cold environment had a higher milk intake for a given body weight than the mice in the warm. If the two groups in the cold were regressed on to a common milk intake there would be no difference between their weights. So the difference between immigrant and Eskimo in the weight of the pups aged 1Ø days was due mainly to differences in milk intake. There was no difference that could be attributed to differences in growth potential.

6.4 Body Composition of pups aged 1Ø days

Fat and water content of pups aged 1Ø days are given in Table 6.7. The litters had been reduced to 5 at birth as were the litters used for the experiments on milk intake. Within-litter variance was again less than between-litter variance for each class ($P<Ø.ØØ1$) so litter means were used as replicates.

Eskimos were heavier than the other classes and had a higher percentage fat. Percentage water of the Eskimos was lower than the other classes. This corresponded to the higher values for percentage fat since percentage fat is negatively correlated with percentage water (4.2.3). There were no differences between the controls and immigrants. The difference between the 1Ø day weights of Eskimos and the two control classes was not due only to differences in fat content. The lean weight of the Eskimo pups was also greater than that of the control and immigrant pups.

Percentage fat was correlated with body weight for all classes (but $P=Ø.Ø9$ for Eskimos) but the correlations were not strong (Table 6.8). Regression of percentage fat on body weight for each class shows that Eskimos had a higher proportion of fat in their bodies than either controls or immigrants when regressed on to the population mean weight (Fig. 6.3).

6.5 Discussion

Although the method of measuring milk intake had flaws (3.2.3.3.1), the results are consistent and resemble those of others who used different techniques (Hanrahan and Eisen, 1970; Baverstock and Elhay, 1978). These authors used direct milking and a tritiated water method, respectively. Their estimates, however, were much lower than those reported by Jara-Amonte and White (1972) who used test feeding.

The figures for the milk intake of the controls resemble those of Rath and Thenen (1979). The mean weight of their laboratory mice at 10 days was, however, 6.3 g whereas that of the controls in this study was 5.3 g.

The accuracy of the method used here could be improved if, immediately after the end of the experiment, one measured the water content of the milk of the mother. Milk intake could then be calculated from the actual water fraction rather than the class average.

The ability of Eskimo mothers to provide much richer milk than the control mothers was evidently an adaptation to cold developed over many generations. The milk of the immigrants (first generation in the cold) had slightly more fat than control milk; but the main difference was between the Eskimo and the other two classes. Highly calorific milk presumably has advantages in a cold environment: extra calories are needed for heat production; and rapid growth and fat deposition probably enhance the pups resistance to cold. The higher protein content of Eskimo milk also matched the need for rapid growth.

In contrast with the findings on milk composition, where the main differences were between mouse types, those on milk intake showed a greater effect of temperature. Pups in the cold drank about 50% more milk than did those

in the warm. The milk consumption of the 1st generation control mice in the cold was similar to that of the 11th generation Eskimo mice. This finding suggests that the greater milk consumption in the cold was a short-term ontogenetic response to cold. This was, of course, expected. Adult laboratory mice transferred to -3° C eat 7Ø% more than controls at 21 C (Barnett and Little, 1965).

Although Eskimo and immigrant pups did not differ in their relative milk intake, Eskimo mothers did produce more milk than control mothers. This raises a question central in any discussion of maternal-offspring relationships. Did the Eskimo females produce more milk because their pups were bigger, or were their pups bigger because the mother produced more milk? From the moment of conception there is a continuing interaction between mother and young. At birth the size and strength of the pup influences the amount of milk it receives, and the milk intake influences its size and strength. Hence the weight of the pup at 1Ø days depends on the amount of milk available (a phenotypic characteristic of the mother), on its growth potential and on complex interactions between the two; in addition there are outside environmental influences. In an unmanipulated situation such as that of the present experiment there is no way of distinguishing between these factors. For a complete comparison of the milk yields of two classes of females, standard foster litters are needed, uniform in birth weight, growth potential and behaviour.

Nagai and Sakar (1978) used mixed cross fostering sets to equalize mean growth potential of the nursed litter when comparing the milk yield of 4 lines selected for different traits. Unfortunately they used a test weighing technique of measuring milk production involving a 6 hour separation period which has serious drawbacks (3.2.3.3.1). They were, however, able to demonstrate differences in milk yield between classes that could be

definitely attributed to differences in the capacity of the mother rather than differences in the growth potential of the young.

Although there was no correlation of Eskimo maternal weight with pup weight, the relationship may still have been present but concealed. For instance, variation in maternal weight was probably due primarily to fat. The fat content of females was not recorded but Eskimo males had large variations in amount of fat (4.3.2.3). There is a direct relationship between milk yield and amount of mammary tissue (Nagai and Sarkar, 1978; Hanwell and Peaker, 1977) and the amount of mammary tissue is likely to be more closely related to fat free body weight than to total body weight. So variations in weight due largely to variations in fat could mask the relationship between maternal weight and pup weight.

It is interesting that immigrants and Eskimos, which drank the same amount of milk, were the same weight when presumably the Eskimos were receiving milk with a higher calorific value. The extra calories probably went into fat deposition, since the Eskimos had a higher proportion of body fat.

The weight of young at 1Ø or 12 days is frequently used as a measure of milk production. Nagai and Sakar (1978) report a strong correlation between 12 day litter weight and milk yield. Hanrahan and Eisen (197Ø) also report a correlation between 12 day litter weight and milk yield, although it was fairly small, probably owing to their method of measuring milk yield. In the present study a strong correlation between milk intake and 1Ø day pup weight was found. Conversely, within a given temperature, milk intake was a fairly good predictor of 1Ø day pup weight. So the use of 1Ø or 12 day litter weight as a measure of milk production of the mother is justified.

7. GENERAL DISCUSSION

This thesis is concerned primarily with problems of adaptation, in particular the gradual change observed in a population during several generations in a new environment. This is in a sense a study in micro-evolution. It is therefore important to make a distinction between ontogenetic and phylogenetic adaptation. Ontogenetic adaption to cold by mice requires principally an increase in metabolic rate and behavioural changes (see reviews by Barnett, 1965, 1973). Phylogenetic adaptation is a property of a population and involves gradual changes over many generations. The distinction is illustrated by the experiments on milk. There was an ontogenetic change in the amount secreted: mice introduced to the cold immediately increased their milk production. Change in milk composition, however, was gradual and took several generations.

Although only a small number of mice (15 pairs) founded the cold population and only half of these subsequently bred, this is not atypical of what occurs in nature (see Introduction). The main problem of the experimental design is not the size of the population but the lack of replicate populations. Genetically different populations respond in different ways to the same environmental demands. The only findings suitable for comparison are those of Barnett (1975) discussed in section 4.4. The Scottish wild mice were bred in a colder temperature (-3 C), but in still air; and the breeding population was smaller; but they can be compared. The two populations differed in some respects, particularly in changes in reproductive performance, but there were similarities. Most notable of these similarities was the gradual increase in body size over generations. Increase in body weight and changes in body shape should be advantageous in a cold environment, other things being equal. A smaller surface to volume ratio reduces heat

loss and hence metabolic rate. But the facts do not always correspond. Although mice from cold regions tend to be heavier than those from warm areas (Berry and Jakobson, 1979) individual populations do not conform with a simple negative correlation between body weight and temperature. For example, mice on the sub-Antartic islands, Macquarie and Marion, experience very similar temperatures and other weather conditions yet the mice on Marion are 1Ø% heavier and shorter than those on Maquarie (Berry et al., 1978). Seasonal changes in body weight of small mammals often present a similar discrepancy. The body weights of a mountain population of RATTUS FUSCUPIES (Australian bush rat) are lower in winter than in summer (Stewart and Barnett, 1983). In nature, temperature is only part of a complex environment to which the animal has to adapt. Responses to food availability and predation, for example are likely to modify the adaptive responses to temperature.

In the present experiment where temperature was the only different factor between the two groups, increase in body size was a striking adaptive response to cold. The Eskimo mice were not only bigger than the controls but also fatter. The extra fat was responsible for much but not all the difference in weight between the two groups. The advantages of extra fat in a cold climate are twofold: as insulation and as a source of energy for heat production.

The greater adult body weight of the Eskimo mice in the later generations could have been achieved by a general increase in growth rate over the whole life span or by an increased growth rate only at a specific period.

Eskimo young in utero evidently grew faster than the control young since they were heavier at birth. During the suckling period the growth rate of the Eskimos was also higher than controls. After this time the relative growth of Eskimos and controls was similar, but the mean

weight gain of the Eskimos was, of course, greater. Hence the heavier adult weights of the Eskimos can be attributed largely to the rapid prenatal and preweaning growth.

The rapid preweaning growth in the cold is especially remarkable because so much of the young mouse's energy budget would be spent on heat production. In addition, the young Eskimos put on more fat during this period than did the controls.

During this rapid early growth the mother is the sole source of food. Maternal effects, then, must be involved in the adaptive responses of growth.

The analysis of maternal effects presented in chapter 5 does show that they had considerable influence on body weight and growth. But the story that has emerged is not very clear. From the experiments on milk it seems that the Eskimo young did not differ from the control young in growth potential. The regression of milk weight on pup weight showed that the difference in weight between the two groups in the same temperature was due to the amount of milk they drank. It would, however, be wrong to say that the growth of pups was determined entirely by the amount of milk provided by the mother. Up to a point the pups determine the amount of milk they receive by their demands; but the capacity of the mother is a limiting factor.

In the fostering experiments the control young reared by Eskimos grew no faster than those reared by controls. Although the Eskimo mothers presumably had a potentially greater supply of milk than the control mothers, the control young did not demand it. Yet the Eskimo young reared by control mothers did as badly as the control young. The control mothers were evidently unable to provide sufficient milk for their needs. Yet in the experiments on reciprocal mating the weight of the young was determined by the type of mother irrespective of the genotype of the young she reared. Hybrid young reared by

Eskimo mothers weighed the same as Eskimo young, although presumably the two groups of young differed in growth potential.

Hence the findings of the fostering and the reciprocal mating experiments appear contradictory. But the experiments cannot strictly be compared. The hybrid young were perhaps able to stimulate greater milk production than the control young of the fostering experiment. The hybrid young with Eskimo mothers also had the advantage of an Eskimo prenatal environment and hence a heavier birth weight. Yet some inconsistencies are difficult to explain. For instance, although the hybrids with Eskimo mothers and the pure Eskimos weighed the same at birth and weaning, after weaning the Eskimos grew much faster. The growth potential of the two groups differed. Perhaps the Eskimo young were limited by the capacity of their Eskimo mothers during the lactation period. Maternal performance is likely to be a limiting factor in adaptive growth.

The situation is a very complex one involving continuing interactions between mother and young. For a greater understanding further analytical experiments are needed. Experiments should use an artificial mother with controllable, standardized behavioral responses and milk supply, or standardized young in a cross-fostering complex that includes prenatal fostering (egg transfer).

This study has, however, illustrated the importance of maternal effects. They influence the growth of the young, especially through increased quantity of richer milk in the cold environment. Maternal effects also influence later reproductive performance in a way unrelated to body weight.

Increased rate of growth of the young and larger adult body size can be regarded as reflecting changes in maternal performance. To test whether maternal effects accumulate over the generations, one needs to cross foster

between Eskimos and controls newly introduced to the cold at each of a series of successive generations. In such an experiment Eskimos which have had an Eskimo postnatal environment could be compared to Eskimos which have had at every generation a control mother as their postnatal environment.

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TABLE 3.1 Experimental conditions: nest temperatures
Each nest contained a litter aged 2 days
Mean temperatures, °C with standard errors
N=10 for each class

		ENVIRONMENT		
		Warm	Cold	P<
A. Mid Young	controls	34.2 ±0.5	29.9 ±0.6	0.001
	eskimos	33.9 ±0.3	30.8 ±1.2	0.01
B. Edge of Young	controls	31.9 ±0.6	27.4 ±0.8	0.001
	eskimos	32.9 ±0.4	27.6 ±1.2	0.001
C. Away from Young (2cm)	controls	25.9 ±0.3	14.1 ±0.8	0.001
	eskimos	26.5 ±0.4	14.4 ±1.1	0.001

TABLE 4.1 Change over generations: reproductive performance. Number of fecund and barren pairs; number of litters per pair; means ± standard errors.

GENERATION	FECUND PAIRS		BARREN PAIRS		LITTERS / PAIR	
0	21		12		2.4 ±0.2	
	Controls	Eskimos	Controls	Eskimos	Controls	Eskimos
1	15	9	0	6	4.7 ±0.3	2.7*** ±0.3
2	12	13	3	2	3.1 ±0.4	2.2 ±0.3
3	11	10	4	5	2.6 ±0.4	3.0 ±0.4
4	10	9	5	6	3.3 ±0.3	2.7 ±0.3
5	16	16	4	4	3.9 ±0.4	4.0 ±0.4
6	25	30	1	0	4.6 ±0.3	4.5 ±0.3
7	13	15	1	0	4.7 ±0.4	5.1 ±0.2
8	13	12	2	3	4.6 ±0.3	-
9	13	14	2	1	3.9 ±0.4	4.5 ±0.3
10	9	16	0	0	3.4 ±0.4	4.6* ±0.2

* , *** different from controls: * $P < 0.05$; *** $P < 0.001$

TABLE 4.2 Change over generations: reproductive performance. Reproductive measurements regressed on generations; regression coefficients and slopes.

MEASURE	WARM ENVIRONMENT r	slope	COLD ENVIRONMENT r	slope
Young born/pair	0.238*	1.06	0.509***	1.93
Young weaned/pair	0.048	0.19	0.450***	1.77
Percentage young weaned/pair	0.163	1.06	0.254**	2.41
Litters born/pair	0.095	0.05	0.632***	0.28
Litter size	0.001	0.00	0.056	0.05
Mating to first litter, days	0.134	0.95	0.435***	-4.50
Parturition intervals, days	0.087	0.29	0.215*	-0.68
Young at 3 wks, g	0.165***	0.09	0.486***	0.23

* P< 0.05
** P< 0.01
*** P< 0.001

TABLE 4.3 Change over generations: reproductive performance.
Litter size at birth; mean number of young per litter
± standard error.
(Numbers of litters in brackets.)

GENERATION	WARM ENVIRONMENT	COLD ENVIRONMENT		
	litter size	litter size	litter size: surviving litters	litter size: non-surviving litters
0	5.7(48) ±0.2			
1	6.2(71) ±0.2	6.0(24) ±0.3	6.3(15) ±0.4	5.3(9) ±0.4
2	6.1(37) ±0.3	6.1(29) ±0.3	6.9(14) ±0.5	5.4++(15) ±0.3
3	5.2(28) ±0.3	6.0(28) ±0.3	6.5(20) ±0.4	5.0+(8) ±0.6
4	5.8(32) ±0.3	5.5(24) ±0.4	5.9(15) ±0.4	4.9(9) ±0.7
5	6.0(59) ±0.2	5.5(56) ±0.3	5.7(41) ±0.4	5.0(15) ±0.6
6	5.8(70) ±0.2	5.3(68) ±0.3	6.1(43) ±0.4	3.8+++(25) ±0.4
7	6.2(52) ±0.3	6.7(76) ±0.3	7.1(56) ±0.3	5.6+(20) ±0.6
8	6.7(60) ±0.3	7.0(32) ±0.6	-	-
9	6.0(51) ±0.3	6.9(63) ±0.4	7.0(57) ±0.4	5.3(6) ±1.0
10	5.3(31) ±0.3	6.1*(74) ±0.3	6.3(64) ±0.3	4.3(10) ±0.4

* different from controls $P < 0.05$

\+ , ++ , +++ } different from surviving litters { $P < 0.05$; $P < 0.01$; $P < 0.001$.

TABLE 4.4 Change over generations: reproductive performance. Total number of young born and weaned per pair and percent young reared to weaning per pair; means ± standard errors.

GENERATION	TOTAL YOUNG BORN		TOTAL YOUNG WEANED		PERCENT YOUNG WEANED	
0	14.0 ±2.3		12.1 ±2.3		77.2 ±0.8	
	Controls	Eskimos	Controls	Eskimos	Controls	Eskimos
1	-	15.8 ±2.5	30.2 ±2.3	10.1*** ±3.0	-	50.9 ±14.7
2	18.7 ±3.0	13.6 ±2.1	17.8 ±3.1	6.7** ±2.0	87.8 ±8.1	41.3** ±10.3
3	13.2 ±3.1	18.0 ±2.5	11.9 ±2.3	12.7 ±1.5	91.1 ±4.9	75.2* ±5.7
4	19.5 ±2.0	14.7 ±2.4	18.4 ±2.1	8.9** ±1.6	94.5 ±2.8	66.0** ±7.9
5	23.5 ±3.1	21.8 ±2.9	21.7 ±2.9	14.2 ±2.7	87.0 ±4.8	64.3* ±8.0
6	27.1 ±2.7	26.4 ±3.2	-	-	-	-
7	29.3 ±4.0	33.9 ±2.8	28.5 ±4.3	24.1 ±4.2	92.2 ±5.5	68.8* ±8.5
8	30.7 ±3.2	-	30.0 ±3.0	-	98.3 ±0.7	-
9	23.6 ±3.8	31.7 ±3.9	23.5 ±3.8	22.3 ±3.8	99.7 ±0.3	71.2** ±5.8
10	18.1 ±2.1	28.0* ±2.8	16.6 ±2.2	23.5 ±3.0	91.7 ±4.1	80.7 ±4.2

*, **, *** different from controls: $P < 0.05$, $P < 0.01$, $P < 0.001$

TABLE 4.5 Change over generations: reproductive performance. Correlation of litter size and parity with 3-week weight. 3-week weight - mean individual weight per litter.
N - number of litters.

GENERATION	N	CORRELATION OF 3-WEEK WEIGHT WITH (a) litter size	(b) parity	CORRELATION OF litter size with parity
WARM ENVIRONMENT				
2	11	-0.63*	0.42	-0.12
3	15	-0.48	0.20	0.25
4	17	-0.19	-0.05	0.40
5	19	0.07	-0.03	0.52*
6	15	0.34	0.35	0.03
7	24	-0.12	0.10	0.49**
COLD ENVIRONMENT				
2	9	0.21	0.55	0.40
3	12	0.02	-0.34	-0.28
4	12	-0.04	0.03	0.20
5	17	0.10	0.18	0.06
6	14	-0.11	0.76***	0.05
7	45	-0.34*	0.22	0.49***
10	43	-0.43**	0.18	0.22
11	20	-0.40	0.25	0.22

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$.

TABLE 4.6 Change over generations: body measurements and organ weights of males aged 30 weeks regressed on generation. Number of animals in brackets

	Controls (100)		Eskimos (141)		Difference between slopes
	r	slope	r	slope	P<
Body weight,g	0.37***	0.41	0.65***	0.94	0.01
Body length,mm	0.08	0.17	0.51***	0.96	0.001
Tail length,mm	0.05	0.11	0.41	0.97	0.001
Tail length/ body length	-0.03	-0.06	0.13	0.28	-
Kidney weight,mg	0.21*	4.7	0.60***	17.5	0.01
Kidney weight, g/100g	0.06	0.01	0.07	0.01	-
Adrenal weight,mg	-0.20*	-0.10	0.47***	-0.29	0.01

* P< 0.05
*** P< 0.001

TABLE 4.7 Change over generations: correlation of body weight with body length and organ weight. (Number of mice in brackets.)

	Controls (100)		Eskimos (141)		Difference between slopes
	r	slope	r	slope	P <
Body length, mm	0.54***	1.04	0.79***	1.07	-
Kidney weight, mg	0.47***	9.89	0.80***	16.80	0.01
Adrenal weight, mg	-0.19	-0.09	0.37**	-0.16	

** P < 0.01
*** P < 0.001

Partial correlation coefficient of adrenal weight with body weight, controlling for generation. (Number of animals in brackets.)

Control (100)	-0.127
Eskimos (141)	-0.068

TABLE 4.8 Mice transferred to the new environment compared with indigenes: reproductive performance.
Number of breeding pairs and number of litters per pair; means ± standard errors

GENERATION	NO. FECUND PAIRS		NO. BARREN PAIRS		LITTERS/PAIR	
	Controls	Eskimos	Controls	Eskimos	Controls	Eskimos
WARM						
5	20	20	4	0	3.9 ±0.4	4.5 ±0.1
6	26	26	1	3	4.6 ±0.3	5.0 ±0.4
7	14	15	1	0	4.7 ±0.4	4.5 ±0.4
COLD						
5	20	20	7	4	2.4+ ±0.3	4.0** ±0.4
6	24	30	3	0	3.0+++ ±0.3	4.5** ±0.3
7	16	15	3	0	4.6 ±0.3	5.1 ±0.2
9	15	15	4	1	2.8 ±0.2	4.5*** ±0.3
10	16	16	1	0	3.1 ±0.3	4.6*** ±0.2
11	10	10	0	1	3.6 ±0.5	5.1* ±0.3

*, **, *** different from controls in the cold: $P < 0.05$, $P < 0.01$, $P < 0.001$

\+, +++ different from controls in the warm: $P < 0.05$, $P < 0.001$

TABLE 4.9 Mice transferred to the new environment compared with indigenes: reproductive performance.
Total number young born and weaned per pair and percent young reared to weaning per pair
means ± standard errors.

GENERATION	TOTAL YOUNG BORN		TOTAL YOUNG WEANED		PERCENT YOUNG REARED	
	Controls	Eskimos	Controls	Eskimos	Controls	Eskimos
WARM						
5	23.5 ±3.1	26.6 ±2.5	21.7 ±2.9	22.7 ±2.3	87.0 ±6.8	86.5 ±4.2
6	27.1 ±3.1	27.8 ±3.0	-	-	-	-
7	29.3 ±4.0	29.1 ±3.1	28.5 ±4.3	26.8 ±3.0	92.2 ±5.5	93.6 ±3.2
COLD						
5	15.9+ ±3.0	21.8 ±2.9	11.3++ ±2.0	14.2+ ±2.7	69.9+ ±7.9	64.3+ ±9.0
6	18.2+ ±2.5	26.4+ ±3.2	-	-	-	-
7	24.6 ±2.7	33.9* ±2.8	18.3+ ±2.5	24.1 ±4.2	73.8+ ±6.6	68.8 ±8.5
9	17.1 ±1.4	31.7** ±3.9	9.1 ±1.0	22.3** ±3.8	55.1 ±5.9	71.2* ±3.8
10	17.7 ±2.2	28.0** ±2.8	9.0 ±2.0	23.5*** ±3.0	47.8 ±8.1	80.7* ±4.2
11	18.5 ±3.9	30.3* ±4.1	14.3 ±4.3	21.1 ±6.0	63.4 ±12.4	62.3 ±14.1

*, **, *** different from controls in the cold: $P < 0.05$, $P < 0.01$, $P < 0.001$

\+, ++ different from same class in warm: $P < 0.05$, $P < 0.01$

TABLE 4.10 Mice transferred to the new environment compared with indigenes: reproductive performance.
Litter size at birth; means ± standard errors; Percent loss of whole litters; total no. litters per class.
N - number of litters

GENERATION	LITTER SIZE				PERCENT LOSS WHOLE LITTERS	
	N	Controls	N	Eskimos	Controls	Eskimos
WARM						
5	59	6.0 ±0.2	65	6.1 ±0.3	5	8
6	70	5.8 ±0.2	49	5.0 ±0.3	4	0
7	52	6.2 ±0.3	65	6.5 ±0.2	4	3
COLD						
5	24	6.6 ±0.4	56	5.5* ±0.3	21	28
6	42	5.9 ±0.3	68	5.3 ±0.3	34	27
7	60	5.3 ±0.3	76	6.7* ±0.3	20	25
9	31	6.1 ±0.3	63	6.9 ±0.4	16	10
10	46	5.8 ±0.3	74	6.1 ±0.3	48	14
11	36	5.2 ±0.4	44	5.9 ±0.4	25	36

* different from controls $P < 0.05$

TABLE 4.11 Transferred mice and indigenes: reproductive performance.
Weight of young at 3 weeks.
Two way analysis of variance.
Factors - temperature: warm or cold
mouse type : control or eskimo

Source of variation	DF	Mean Square	F	P<
Generation 5				
temperature	1	228.5	367.5	0.001
mouse type	1	84.1	135.3	0.001
interaction	1	12.4	19.9	0.001
residual	555	0.6		
total	558	1.2		
Generation 6				
temperature	1	156.1	111.4	0.001
mouse type	1	99.4	71.0	0.001
interaction	1	0.7	0.5	0.5
residual	352	1.4		
total	355	2.1		
Generation 7				
temperature	1	11.2	10.1	0.001
mouse type	1	130.3	118.4	0.001
interaction	1	16.0	14.5	0.001
residual	979	1.1		
total	982	1.3		

TABLE 4.12 Mice transferred to the new environment compared with indigenes; body measurements of males aged 30 weeks. Means ± standard errors.

GENERATION	NO. MICE		BODY WEIGHT, g		BODY LENGTH, mm	
	Con.	Eskimos	Controls	Eskimos	Controls	Eskimos
WARM						
5	18	11	20.6 ±0.3	21.6 ±0.5	89.2 ±0.7	92.2* ±0.9
6	19	21	21.2 ±0.7	21.4 ±0.5	91.4 ±0.7	91.7 ±0.8
7	12	16	22.1 ±0.8	23.1 ±0.8	90.8 ±1.2	93.4 ±1.0
COLD						
5	17	17	23.3+++ ±0.5	23.1 ±0.6	92.5+ ±1.1	91.8 ±0.7
6	21	21	21.0 ±0.6	25.3***+++ ±0.8	90.1 ±0.9	94.8***+ ±0.9
7	14	14	22.0 ±0.6	26.4***+++ ±0.8	91.8 ±0.8	97.2***+ ±0.9
9	14	14	23.8 ±0.9	27.7** ±0.8	92.3 ±0.9	97.9*** ±0.6
10	12	15	21.7 ±0.7	27.4*** ±0.6	88.3 ±1.1	96.0*** ±0.8
11	9	9	23.2 ±1.0	28.1** ±1.3	89.0 ±1.0	95.2** ±1.7

*, **, *** different from controls in the same temperature: P < 0.05, P < 0.01, P < 0.001

+, ++, +++ different from same mouse type in the warm: P < 0.05, P < 0.01, P < 0.001

TABLE 4.13 Mice transferred to the new environment compared with indigenes; tail measurements of males aged 30 weeks. Means ± standard errors.

GENERATION	NO. MICE		TAIL LENGTH, mm		TAIL LENGTH/BODY LENGTH	
	Con.	Eskimos	Controls	Eskimos	Controls	Eskimos
WARM						
5	18	11	78.3 ±0.7	71.3*** ±1.8	87.9 ±0.9	77.3*** ±2.0
6	19	21	83.4 ±0.7	85.9* ±0.9	91.3 ±0.8	93.7* ±0.7
7	12	16	79.8 ±0.9	85.5*** ±1.1	88.1 ±1.1	91.6* ±1.0
COLD						
5	17	17	79.2 ±0.9	69.2*** ±1.6	85.6 ±1.3	75.3*** ±1.6
6	21	21	68.5+++ ±1.3	71.2+++ ±1.5	75.9+++ ±1.1	75.1+++ ±1.3
7	14	14	67.8+++ ±1.3	73.2**++ ±1.3	73.9+++ ±1.1	75.3+++ ±1.6
9	14	14	80.2 ±0.7	72.2*** ±1.5	87.0 ±1.0	73.8 ±1.6
10	12	15	68.7 ±1.1	74.1** ±1.5	77.9 ±1.3	77.2 ±1.6
11	9	9	64.2 ±2.2	71.7* ±1.4	72.1 ±2.2	75.4 ±1.8

*, **, *** different from controls in the same temperature — $P < 0.05$, $P < 0.01$, $P < 0.001$

+++ different from same mouse type in the warm — $P < 0.001$

TABLE 4.14 Transferred mice and indigenes of generations 6 and 7: body weight and body and tail length of males aged 30 weeks.
Three way analysis of variance.
Factors - temperature: warm or cold
mouse type: control or eskimo
mother type: generation 5 mother or generation 6 mother.

Source of variation	DF	Mean Square	F	P <
Body weight,g				
temperature	1	112.2	12.7	0.001
mouse type	1	217.5	24.6	0.001
mother type	1	43.3	4.9	0.03
interaction				
temp x mouse	1	124.4	14.1	0.001
residual	133	8.8		
total	140	11.8		
Body length,mm				
temperature	1	80.8	5.6	0.02
mouse type	1	358.6	24.8	0.001
mother type	1	57.1	4.0	0.05
interaction				
temp x mouse	1	123.1	8.5	0.005
residual	133	14.4		
total	140	18.3		
Tail length,mm				
temperature	1	6663.6	255.6	0.001
mouse type	1	504.7	19.4	0.001
mother type	1	11.6	0.4	0.6
interaction				
temp x mouse	1	0.1	0.001	1.0
residual	133	26.1		
total	140	78.8		

TABLE 4.15 Mice transferred to the new environment compared with indigenes: organ weights of males aged 30 weeks.
Means ± standard errors
N - number of mice.

GENERATION	N		KIDNEY WEIGHT mg		RELATIVE KIDNEY WEIGHT, g/100g		ADRENAL WEIGHT mg	
	Con	Esk	Con	Esk	Controls	Eskimos	Controls	Eskimos
WARM								
5	18	11	323.6 ±12.5	320.6 ±11.9	1.54 ±0.06	1.48 ±0.04	4.37 ±0.29	4.32 ±0.35
6	19	21	339.5 ±13.1	328.4 ±10.7	1.61 ±0.06	1.54 ±0.04	4.22 ±0.14	4.24 ±0.23
7	12	16	329.2 ±14.0	398.8* ±19.4	1.50 ±0.05	1.72** ±0.05	4.11 ±0.29	3.70 ±0.34
COLD								
5	13	17	429.8+++ ±26.0	404.6+++ ±14.4	1.84+ ±0.9	1.75+++ ±0.04	4.90 ±0.22	4.04 ±0.39
6	22	21	381.6+ ±10.8	477.5$^{+++}_{***}$ ±14.0	1.82++ ±0.04	1.91+++ ±0.08	4.32 ±0.23	4.34 ±0.26
7	16	14	395.4++ ±13.4	483.9$^{++}_{***}$ ±17.5	1.80+++ ±0.03	1.84 ±0.05	5.25+ ±0.29	4.28 ±0.45
9	13	14	386.1 ±13.1	492.3*** ±14.4	1.64 ±0.07	1.78 ±0.05	4.25 ±0.24	4.16 ±0.22
10	12	15	417.5 ±18.0	495.5*** ±12.6	1.93 ±0.06	1.81 ±0.03	4.23 ±0.40	3.88 ±0.27
11	9	9	429.3 ±19.1	487.7* ±20.2	1.85 ±0.04	1.74 ±0.05	4.37 ±0.30	3.61 ±0.35

*, **, *** different from controls in same temperature: $P < 0.05$; $P < 0.01$; $P < 0.001$

+, ++, +++ different from same mouse type in the warm: $P < 0.05$; $P < 0.01$; $P < 0.001$.

TABLE 4.16 Mice transferred to the new environment compared with indigenes: body composition of males aged 30 weeks or older.
Absolute and relative body fat, fat free body weight:
means ± standard errors.

Generation	numbers		body fat, g		body fat, %		fat free body weight, g	
	cont.	esk.	controls	eskimos	controls	eskimos	controls	eskimos
Warm								
5	18	13	1.33 ±0.13	1.50 ±0.20	8.6 ±0.8	9.3 ±0.9	18.8 ±0.4	19.6 ±0.4
7	12	16	1.68 ±0.29	1.66 ±0.17	9.2 ±1.4	9.2 ±0.8	20.0 ±0.7	21.0 ±0.7
Cold								
5	13	17	1.64 ±0.16	1.94 ±0.25	9.5 ±0.9	11.0 ±1.2	21.0+++ ±0.5	20.5 ±0.4
7	16	14	2.27 ±0.16	3.32*++ ±0.49	13.5+ ±0.8	16.6++ ±2.1	19.0 ±0.5	21.9*** ±0.6
11	9	9	2.53 ±0.40	3.30 ±0.61	14.6 ±1.7	15.3 ±2.0	19.7 ±0.7	23.7*** ±0.6

* , *** different from controls in the cold: P<0.05, P<0.001

++ , +++ different from controls in the warm: P<0.01, P<0.001

TABLE 5.1 Fostered Mice: growth
Body weight at birth
Three way analysis of variance.
Factors - temperature: warm or cold
true mother: eskimo or control
foster mother: eskimo or control

Source of variation	DF	Mean Square	F	P<
temperature	1	0.062	4.2	0.05
true mother	1	0.314	21.3	0.001
foster mother	1	0.041	2.8	0.1
interaction				
true mother x foster mother	1	0.00	0.02	0.9
residual	107	0.015		
total	114	0.018		

TABLE 5.2 Fostered Mice: growth

Body weight at 10 days

Three way analysis of variance.

Factors - temperature: warm or cold

true mother: eskimo or control

foster mother: eskimo or control

Source of variation	DF	Mean Square	F	P<
temperature	1	2.38	4.6	0.04
true mother	1	8.32	16.1	0.001
foster mother	1	8.45	16.3	0.001
interaction				
true mother x foster mother	1	1.94	3.7	0.06
residual	106	0.52		
total	113	0.69		

TABLE 5.3 Fostered Mice: growth

Body weight at 21 days

Three way analysis of variance.

Factors - temperature: warm or cold

true mother: eskimo or control

foster mother: eskimo or control

Source of variation	DF	Mean Square	F	P <
temperature	1	25.5	30.4	0.001
true mother	1	17.6	21.0	0.001
foster mother	1	9.6	11.4	0.002
interaction				
true mother x foster mother	1	4.7	5.6	0.02
residual	105	0.8		
total	112	1.3		

TABLE 5.4 Fostered Mice: growth

Body weight at 10 weeks

Three way analysis of variance.

Factors - temperature: warm or cold

true mother: eskimo or control

foster mother: eskimo or control

Source of variation	DF	Mean Square	F	P<
temperature	1	7.8	1.4	0.3
true mother	1	77.1	14.1	0.001
foster mother	1	7.1	1.3	0.3
interaction				
true mother x foster mother	1	16.6	3.0	0.09
residual	91	5.5		
total	98	6.3		

TABLE 5.5 Fostered Mice: growth

Body weight at 16 weeks

Three way analysis of variance.

Factors - temperature: warm or cold

true mother: eskimo or control

foster mother: eskimo or control

Source of variation	DF	Mean Square	F	P<
temperature	1	1.8	0.3	0.6
true mother	1	95.6	17.0	0.001
foster mother	1	10.3	1.8	0.2
interaction				
true mother x foster mother	1	7.6	1.4	0.3
residual	96	5.6		
total	103	6.5		

TABLE 5.6 Fostered Mice: reproductive performance
No litters, young born/weaned, rearing success and interval, mating to first litter per pair.
means ± standard errors

	Controls	Controls reared by eskimo	Eskimos	Eskimos reared by controls
WARM				
no. fecund pairs (barren pairs in brackets)	13(1)	15(1)	15(0)	15(1)
no. litters/pair	4.7 ±0.4	4.9 ±0.3	4.5 ±0.4	4.9 ±0.3
young born/pair	29.3 ±4.0	30.2 ±3.1	29.1 ±3.1	26.3 ±2.1
young weaned/pair	28.5 ±4.3	28.6 ±3.4	26.8 ±3.0	25.6 ±2.2
young reared to weaning,%	92.2 ±5.5	93.6 ±3.6	93.6 ±3.2	96.9 ±1.1
interval, mating to birth first litter, days	35.1 ±7.4	38.7 ±6.4	43.2 ±6.1	40.6 ±7.7
COLD				
no. fecund pairs (barren pairs in brackets)	13(3)	12(4)	15(0)	16(1)
no. litters/pair	4.6 ±0.3	3.8 ±0.5	5.1 ±0.2	5.1 ±0.3
young born/pair	24.6 ±2.7	24.6 ±5.1	33.9 ±2.8	30.6 ±2.9
young weaned/pair	18.3 ±2.5	18.8 ±5.8	24.1 ±4.2	22.5 ±3.8
young reared to weaning,%	73.8 ±6.6	65.8 ±9.9	68.8 ±8.5	70.6 ±7.4
interval mating to birth of first litter, days	49.1 ±6.7	78.8 ±13.6	37.8 ±6.6	39.6 ±7.6

no differences due to foster parent

for comparisons of eskimos and controls see Tables 4.8 and 4.9 and Fig 4.3.

TABLE 5.7 Fostered Mice: reproductive performance

Litter size at birth, loss of whole litters per class.

means ± standard errors

	Controls	Controls reared by eskimos	Eskimos	Eskimos reared by controls
WARM				
Number of litters	52	70	6	6
Litter size at birth	6.2 ±0.3	6.1 ±0.3	6.5 ±0.2	5.3*** ±0.3
Loss of whole litters,%	4	1	3	2
COLD				
Number of litters	60	38	76	74
Litter size at birth	5.3 ±0.3	6.4* ±0.4	6.7 ±0.3	6.0 ±0.3
Loss of whole litters,%	20	18	25	26

* } different from within-class fostered group { P<0.05

*** } { P<0.001

for comparisons of eskimos and controls see Table 4.10.

TABLE 5.8 Fostered Mice: reproductive performance
Weight of young at 3 weeks in the cold environment
Two way analysis of variance.
Factors - true mother : eskimo or control
foster mother : eskimo or control

Source of variation	DF	Mean Square	F	P<
true mother	1	12.8	12.7	0.001
foster mother	1	2.9	2.9	0.09
interaction	1	8.8	8.7	0.005
residual	138	1.0		
total	141	1.2		

TABLE 5.9 Fostered Mice: reproductive performance
Litter size at birth in the cold environment
Two way analysis of variance.
Factors - true mother : eskimo or control
foster mother : eskimo or control

Source of variation	DF	Mean Square	F	P<
true mother	1	15.0	2.6	0.2
foster mother	1	41.1	7.0	0.01
interaction	1	2.7	0.5	0.5
residual	244	5.9		
total	247	6.1		

TABLE 5.10 Fostered Mice: growth

Body weight and body and tail length of males aged 16 weeks

means ± standard errors

	Controls	Controls reared by eskimos	Eskimos	Eskimos reared by controls
WARM				
number of mice	26	21	20	28
body weight, g	20.0 ±0.4	21.4 ±0.7	22.1++ ±0.6	20.4* ±0.4
body length, mm	87.5 ±0.6	90.1* ±0.8	90.3++ ±0.9	88.8 ±0.6
tail length, mm	76.8 ±0.6	78.3 ±0.8	83.6+++ ±0.9	79.6 ±0.8
tail/body length x100	87.8 ±0.7	86.9 ±0.8	92.6+++ ±1.1	89.8* ±0.8
COLD				
number of mice	14	18	17	19
body weight, g	17.5 ±0.5	18.4 ±0.4	21.9+++ ±0.5	21.1 ±0.7
body length, mm	83.3 ±0.7	84.8 ±0.7	91.2+++ ±0.7	88.8* ±0.9
tail length, mm	61.2 ±1.3	63.9 ±1.2	67.8 ±0.6	64.2* ±1.5
tail/body length x100	73.5 ±1.4	75.5 ±1.4	74.4 ±0.8	72.2 ±1.4

* different from within class fostered group $P<0.05$

++ different from controls $P<0.01$

+++ different from controls $P<0.001$

TABLE 5.11 Fostered Mice: growth

Body weight and body and tail length of males aged 30 weeks or older.

means ± standard errors

	Controls	Controls reared by eskimos	Eskimos	Eskimos reared by controls
WARM				
number of mice	12	16	20	13
body weight, g	22.1 ±0.8	21.9 ±1.0	23.1 ±0.8	23.4 ±0.8
body length, mm	90.8 ±1.2	92.7 ±1.6	93.4 ±1.0	94.6 ±1.0
tail length, mm	79.8 ±0.9	82.1 ±1.1	85.5 ±1.1	84.0 ±1.1
tail/body length x100	88.1 ±1.1	88.7 ±0.9	91.6 ±1.0	88.9 ±1.2
COLD				
number of mice	14	16	14	16
body weight, g	22.0 ±0.6	22.5 ±0.7	26.4 ±0.8	23.1** ±0.7
body length, mm	91.8 ±0.8	92.6 ±0.7	97.2 ±0.9	94.3* ±1.0
tail length, mm	67.8 ±1.3	69.6 ±1.9	73.2 ±1.3	70.8 ±1.6
tail/body length x100	73.9 ±1.1	75.2 ±2.1	75.3 ±1.0	75.0 ±1.4

* different from within class fostered group, $P<0.05$

** different from within class fostered group, $P<0.01$

for comparisons of eskimo and control see Table 4.12 and 4.13.

TABLE 5.12 Fostered Mice: growth

Body weight and body and tail length of males aged 16 weeks

Three way analysis of variance.

Factors - temperature : warm or cold
true mother : control or eskimo
foster mother : control or eskimo

Source of variation	DF	Mean Square	F	P<
Body weight, g				
temperature	1	53.1	8.7	0.005
true mother	1	128.3	20.9	0.001
foster mother	1	61.8	10.1	0.003
interaction				
temp x true mother	1	87.0	14.2	0.001
residual	155	6.1		
total	162	7.8		
Body length, mm				
temperature	1	167.8	14.8	0.001
true mother	1	352.4	31.1	0.001
foster mother	1	153.4	13.5	0.001
interaction				
temp x true mother	1	273.1	24.1	0.001
residual	155	11.3		
total	162	16.4		
Tail length, mm				
temperature	1	9200.9	492.7	0.001
true mother	1	554.9	29.7	0.001
foster mother	1	341.1	18.3	0.001
interaction				
temp x true mother	1	3.4	0.2	0.7
residual	155	18.8		
total	162	78.2		

TABLE 5.13 Fostered mice: growth

Body weight and body and tail length of males aged 30 weeks or older.

Three way analysis of variance.

Factors - temperature : warm or cold
true mother : control or eskimo
foster mother : control or eskimo

Source of variation	DF	Mean Square	F	P<
Body weight, g				
temperature	1	23.5	2.5	0.2
true mother	1	106.4	11.5	0.002
foster mother	1	21.1	2.3	0.2
residual	111	9.2		
total	118	10.5		
Body length, mm				
temperature	1	34.6	2.0	0.2
true mother	1	244.4	14.4	0.001
foster mother	1	36.6	2.2	0.2
residual	111	17.0		
total	118	19.2		
Tail length, mm				
temperature	1	4621.3	168.0	0.001
true mother	1	359.1	13.0	0.001
foster mother	1	119.7	4.4	0.04
residual	111	27.5		
total	118	70.9		

TABLE 5.14 Fostered Mice: organ weights

Kidney and adrenal weights of males ages 16 weeks

means ± standard errors

	Controls	Controls reared by eskimos	Eskimos	Eskimos reared by controls
WARM				
number of mice	26	21	20	28
kidney weight, mg	311.7 ±11.3	367.4** ±12.4	341.7+ ±13.4	344.7 ±11.0
relative kidney weight, g/100g	1.56 ±0.04	1.72** ±0.04	1.55 ±0.06	1.69* ±0.06
adrenal weight, mg	4.24 ±0.24	4.50 ±0.15	4.19 ±0.23	3.56 ±0.21
COLD				
number of mice	14	18	17	19
kidney weight, mg	303.5 ±10.6	339.4 ±14.5	380.0+++ ±10.1	372.5 ±13.4
relative kidney weight, g/100g	1.74 ±0.06	1.84 ±0.07	1.74 ±0.03	1.78 ±0.05
adrenal weight, mg	5.30 ±0.19	4.84 ±0.19	3.87+++ ±0.17	3.96 ±0.19

* different from within class fostered group $P<0.05$

** different from within class fostered group $P<0.01$

\+ different from controls $P<0.05$

+++ different from controls $P<0.001$

TABLE 5.15 Fostered Mice: organ weights

Kidney and adrenal weights of males aged 30 weeks or older

means ± standard errors

	Controls	Controls reared by eskimos	Eskimos	Eskimos reared by controls
WARM				
number of mice	12	16	20	13
kidney weight, mg	329.2 ±14.0	341.9 ±16.7	398.8 ±19.4	348.5* ±11.3
relative kidney weight, g/100g	1.50 ±0.05	1.57 ±0.04	1.72 ±0.05	1.50** ±0.05
adrenal weight, mg	4.11 ±0.29	3.85 ±0.27	3.70 ±0.34	3.60 ±0.23
COLD				
number of mice	14	16	14	16
kidney weight, mg	395.4 ±13.4	400.7 ±17.2	483.9 ±17.5	436.0 ±16.7
relative kidney weight, g/100g	1.80 ±0.05	1.79 ±0.05	1.84 ±0.05	1.89 ±0.05
adrenal weight, mg	5.25 ±0.29	4.23** ±0.18	4.28 ±0.45	3.76 ±0.27

* } different from within class fostered group { P<0.05
** } { P<0.01

for comparisons between eskimos and controls see Table 4.15.

TABLE 5.16 Fostered mice: body composition

Body fat of males aged 30 weeks or older

means ± standard errors.

	Controls	Controls reared by eskimos	Eskimos	Eskimos reared by controls
WARM				
number of mice	12	16	20	13
body fat, g	1.68 ±0.29	1.90 ±0.28	1.66 ±0.17	2.42 ±0.48
body fat, %	9.2 ±1.4	10.8 ±1.2	9.2 ±0.8	12.7 ±2.0
fat free body weight,g	20.0 ±0.7	19.5 ±0.8	21.0 ±0.7	20.3 ±0.6
COLD				
number of mice	14	16	14	16
body fat, g	2.27 ±0.16	2.34 ±0.19	3.32 ±0.49	1.88* ±0.28
body fat, %	13.5 ±0.8	13.6 ±0.9	16.6 ±2.1	10.6* ±1.3
fat free body weight, g	19.0 ±0.5	19.4 ±0.6	21.9 ±0.6	20.6 ±0.6

* different from within class fostered group $P<0.05$

for comparisons between eskimos and controls see Figs. 4.14 and 4.15.

TABLE 5.17 Reciprocally mated pairs of their offspring: maternal weights
Body weights of females at time of mating (8-10 weeks)
means ± standard errors

Reciprocally mated pairs

	Controls		Eskimos	
	control mate	eskimo mate	control mate	eskimo mate
number of mice	15	15	15	15
body weight, g	14.1 ±0.5	14.5 ±0.6	16.4*** ±0.3	16.7*** ±0.5

*** different from controls with either mate type $P<0.001$

Offspring of reciprocal matings

	Controls	hybrids with control mothers	hybrids with eskimo mothers	eskimos
number of mice	10	10	10	10
body weight, g	11.8 ±0.6	13.9++ ±0.4	14.9*** ±0.4	16.4*** ±0.6

++ different from mother's class $P<0.01$
*** different from controls $P<0.001$

TABLE 5.18 Reciprocally mated pairs:
reproductive performance
No. litters, young born/weaned, rearing success
and interval from mating to first litter per pair:
Litter size at birth per class
means ± standard errors
Percent loss whole litters per class

	Controls	Eskimos	Eskimo x Control	Control x Eskimo
no. fecund pairs (barren pairs in brackets)	15(1)	16(0)	14(1)	14(1)
no. litters/pair	3.1 ±0.3	4.6++ ±0.2	4.1+ ±0.3	5.0+++ ±0.3
young born/pair	17.7 ±2.2	28.0++ ±2.8	28.5++ ±2.6	32.4+++ ±2.4
young weaned/pair	9.0 ±2.0	23.5++ ±3.0	20.8++ ±3.2	18.4++ ±3.4
young reared to weaning, %	47.8 ±8.1	80.7**++ ±4.7	67.3 ±8.1	53.4 ±7.8
interval, mating to birth of first litter, days	59.9 ±6.1	43.3* ±4.1	39.4+ ±5.0	30.9+++ ±2.1
no. litters/class	46	74	58	70
litter size at birth	5.8 ±0.3	6.1 ±0.3	6.9+ ±0.3	6.5 ±0.2
loss of whole litters, %	48	14	24	39

* } different from control x eskimo { P<0.05
** } { P<0.01

\+ } different from controls { P<0.05
++ } { P<0.01
+++ } { P<0.001

TABLE 5.19 Reciprocally mated pairs: reproductive performance

Total no. young born/pair, percentage young reared.

Two way analysis of variance.

factors - female type : eskimo or control

male type : eskimo or control

Source of variation		DF	Mean Square	F	P<
Total young born/pair					
	female type	1	709.4	7.4	0.01
	male type	1	140.2	1.5	0.3
	interaction	1	856.8	8.9	0.005
	residual	55	96.3		
	total	58	121.3		
Percentage young reared					
	female type	1	1338.5	1.8	0.2
	male type	1	8100.5	10.8	0.003
	interaction	1	226.2	0.3	0.6
	residual	55	747.3		
	total	58	881.4		

TABLE 5.20 Reciprocally mated pairs: reproductive performance.
Correlation of weaning weight (3 weeks) of young with litter size and parity.
Correlation coefficients
n - number of litters

Mated pairs male x female	n	Correlation of weaning weight of young with litter size	with parity
eskimo x eskimo	45	-0.433**	0.278*
control x control	22	0.347	0.009
eskimo x control	36	0.028	0.221
control x eskimo	29	-0.382*	0.391*
pairs with eskimo female	76	-0.409***	0.325**
pairs with control female	60	-0.141	0.130

* P<0.05
** P<0.01
*** P<0.001

TABLE 5.21 Reciprocally mated pairs : body measurements and organ weights of males aged 30 weeks. means ± standard errors

	Eskimos		Controls	
	Control mate	Eskimo mate	Control mate	Eskimo mate
number of mice	14	15	12	15
body weight, g	25.9 ±1.0	27.4 ±0.6	21.7 ±0.7	21.5 ±0.8
body length, mm	93.4 ±0.7	96.0* ±0.8	88.3 ±1.1	86.5 ±1.3
tail length, mm	73.4 ±1.6	74.1 ±1.5	68.7 ±1.1	70.7 ±0.9
tail:body length ratio,%	78.6 ±1.5	77.2 ±1.6	77.9 ±1.3	82.0 ±1.2
kidney weight, mg	464.6 ±14.7	495.5 ±12.6	417.5 ±18.0	390.2 ±14.0
relative kidney weight, g/100g	1.81 ±0.04	1.81 ±0.03	1.93 ±0.06	1.83 ±0.06
adrenal weight, mg	3.37 ±0.18	3.88 ±0.27	4.23 ±0.40	4.29 ±0.21

* difference between eskimo males due to mate type $P<0.05$

TABLE 5.22 Offspring of reciprocal matings : reproductive performance

No. litters, young born/weaned, rearing success and interval from mating to first litter per pair: litter size at birth per class.

means ± standard errors

Percent loss whole litters per class

	Controls	Eskimos	Hybrids with control mother	Hybrids with eskimo mother
no. fecund pairs (barren pairs in brackets)	10(0)	9(1)	8(2)	10(0)
no. litters/pair	3.6 ±0.5	5.1 ±0.3	3.5 ±0.3	5.0* ±0.5
young born/pair	18.5 ±3.9	30.3 ±4.1	20.1 ±2.1	38.9** ±5.2
young weaned/pair	14.3 ±4.3	21.1 ±6.0	11.0 ±1.6	33.6** ±5.4
young reared to weaning, %	63.4 ±12.4	62.3 ±14.1	56.9 ±8.4	86.2* ±4.6
interval mating to birth of first litter, days	65.4 ±15.4	34.1 ±3.6	72.0 ±6.4	40.2* ±10.5
no. litters/class	36	44	28	47
litter size at birth	5.2 ±0.4	5.9 ±0.6	5.8 ±0.3	7.7***++ ±0.3
loss whole litters,%	25	36	36	11

*, **, *** different from other hybrid class ($P<0.05$, $P<0.01$, $P<0.001$)

++ different from class which mother derived $P<0.01$

for comparisons between controls and eskimos see Tables 4.8-10

TABLE 5.23 Offspring of reciprocal matings : reproductive performance.
Correlation of weaning weight (3 weeks) of young with litter size and parity.
Correlation coefficients
n - number of litters

	n	Correlation of weaning weight of young with litter size	with parity
controls	22	0.164	0.455*
eskimos	19	-0.271	0.210
hybrids with control mothers	16	-0.007	0.122
hybrids with eskimo mothers	35	-0.010	0.109

* $P<0.05$

TABLE 5.24 Offspring of reciprocal matings: growth
Body weight and body and tail length of males aged 16 weeks and 30 weeks or older
means ± standard errors

	Controls	eskimos	hybrids with control mothers	hybrids with eskimo mothers
Males ages 16 weeks number of mice	40	47	55	44
body weight, g	18.5 ±0.3	23.9 ±0.4	19.3 ±0.3	22.0***+++ ±0.3
body length, mm	82.2 ±0.5	90.8 ±0.5	84.3+++ ±0.4	87.1***+++ ±0.4
tail length, mm	57.9 ±0.6	67.2 ±0.9	60.9++ ±0.6	65.8*** ±0.8
tail length/ body length	70.4 ±0.8	74.0 ±0.8	72.2 ±0.7	75.6** ±0.8
Males ages 30 weeks or older number of mice	9	9	10	11
body weight, g	23.2 ±1.0	28.1 ±1.3	22.2 ±0.9	23.4++ ±0.6
body length, mm	89.0 ±1.0	95.2 ±1.7	88.9 ±0.9	91.4*+ ±0.5
tail length, mm	64.2 ±2.2	71.7 ±1.4	67.3 ±1.3	70.2 ±0.8
tail length/ body length	72.1 ±2.2	75.4 ±1.8	75.8 ±1.5	76.8 ±0.7

*, **, *** different from other hybrid class ($P<0.05$ +, $P<0.01$ ++, $P<0.001$ +++ different from mother's class $P<0.05$, $P<0.01$, $P<0.001$)

for comparisons between controls and eskimos see Tables 4.12 and 4.13

TABLE 5.25 Offspring of reciprocal matings: organ weights
Kidney and adrenal weights of males aged 16 weeks and 30 weeks or older
means ± standard errors

	Controls	eskimos	hybrids with control mothers	hybrids with eskimo mothers
Males aged 16 weeks number of mice	40	47	55	44
kidney weight,mg	349.3 ±9.2	449.7 ±8.7	368.5 ±6.6	411.5*** +++ ±6.9
relative kidney weight, g/100g	1.89 ±0.03	1.88 ±0.02	1.91 ±0.03	1.88 ±0.03
adrenal weight, mg	3.97 ±0.21	3.66 ±0.14	4.09 ±0.14	4.02 ±0.14
Males aged 30 weeks number of mice	9	9	10	11
kidney weight,mg	429.3 ±19.1	487.7 ±20.2	424.6 ±16.1	442.3 ±12.1
relative kidney weight, g/100g	1.85 ±0.04	1.74 ±0.05	1.92 ±0.05	1.89 + ±0.05
adrenal weight, mg	4.37 ±0.30	3.61 ±0.35	3.64 ±0.36	4.30 ±0.19

*** different from other hybrid class P<0.001
+ ⎫ different from mother's class ⎧ P<0.05
+++ ⎭ ⎩ P<0.001

for comparisons between eskimos and controls see Table 4.15

TABLE 5.26 Offspring of reciprocal matings: body composition
Body fat of males aged 30 weeks or older
means ± standard errors

	Controls	eskimos	hybrids with control mothers	hybrids with eskimo mothers
number of mice	9	9	10	11
body fat, g	2.5 ±0.4	3.3 ±0.6	2.0 ±0.3	2.4 ±0.2
body fat, %	14.6 ±1.7	15.3 ±2.0	11.4 ±1.4	13.3 ±1.1
fat free body weight, g	19.7 ±0.7	23.7 ±0.6	19.6 ±0.7	20.4+++ ±0.4

+++ different from mother's class $P<0.001$

for comparison of eskimos and controls see Table 4.16.

TABLE 6.1 Milk supply: milk composition
means ± standard errors.

	Controls	Immigrants	Eskimos	Cow
No. of replicates	10	10	12	10
Specific gravity	1.03 ±0.002	1.03 ±0.004	1.03 ±0.003	1.03 ±0.002
Total solids mg/100µl	27.6 ±0.5	28.3 ±0.6	38.8***+++ ±1.1	12.9 ±0.1
Water mg/100µl	72.4 ±0.5	71.7 ±0.6	61.2***+++ ±1.1	87.1 ±0.1
Fat mg/100µl	11.1 ±0.3	14.4*** ±0.7	20.4***+++ ±0.6	3.8 ±0.1
Protein mg/100µl	6.5 ±0.2	5.4*** ±0.1	7.9***+++ ±0.2	3.2 ±0.1

*** different from controls P<0.001
+++ different from immigrants P<0.001

TABLE 6.2 Milk supply: 24 hour milk intake of pups aged 10 days
means of litter means ± standard errors.

	Controls	Immigrants	Eskimos
No. of litters	11	10	10
Maternal weight, g	22.4 ±0.8	27.9*** ±1.2	32.4*** ±1.3
Pup weight, g	5.3 ±0.2	5.2 ±0.2	6.2++ ±0.3
Milk intake per pup, g	0.81 ±0.07	1.16*** ±0.06	1.62+++ ±0.06
Milk intake/pup weight	0.15 ±0.001	0.23*** ±0.01	0.26 + ±0.01

* } different from controls { P<0.01
*** } { P<0.001

\+ } different from immigrants { P<0.05
++ } { P<0.01
+++ } { P<0.001

TABLE 6.3 Milk supply: pup body weight and 24 hour milk intake
One way analysis of variance.

	DF	MS	F	P <
Body weight of pups, g				
Controls				
Between litters	10	1.52	17.07	0.001
Within litters	42	0.09		
Total	52			
Immigrants				
Between litters	9	2.81	15.32	0.001
Within litters	39	0.18		
Total	48			
Eskimos				
Between litters	9	3.67	28.90	0.001
Within litters	37	0.13		
Total	46			
Milk intake, g				
Controls				
Between litters	10	0.22	36.63	0.001
Within litters	42	0.01		
Total	52			
Immigrants				
Between litters	9	0.18	16.01	0.001
Within litters	39	0.01		
Total	48			
Eskimos				
Between litters	9	0.19	16.00	0.001
Within litters	37	0.01		
Total	46			

TABLE 6.4 Milk Supply: correlation coefficients for body weights of pups aged 10 days, milk intake and maternal weight
No. of litters in brackets

	Pup body weight with Milk Intake	Pup body weight with Maternal weight	Maternal weight with Milk Intake
Controls (11)	0.798**	0.622*	0.835***
Immigrants (10)	0.626*	0.645*	0.533*
Eskimo (10)	0.639*	0.449	0.182
All Classes (31)	0.698***	0.634***	0.793***

* $P<0.05$
** $P<0.01$
*** $P<0.001$

TABLE 6.5 Milk supply: body weights of pups aged 10 days and their milk intake

Pup weight regressed on milk intake and maternal weight

Multiple regression analysis: estimated values

	Estimate	S.E.	T	P<
Immigrants	1.216	0.831	1.46	
Controls	1.034*	0.300	3.61	0.01
Eskimos	-0.122*	0.330	-0.37	
Milk	1.896	0.557	3.41	0.01
Mother	0.063	0.032	2.00	0.06

* deviation from value of immigrants.

TABLE 6.6 Milk supply: pup body weight and milk intake
Pup body weight regressed on milk intake and maternal weight
Summary of modifications to multiple regression model

	Residual DF	Residual Deviance	Change DF	Change Deviance	Mean Change	Mean deviation ratio *	P<
Initial model constant	20	22.028					
Modifications							
+ class	28	15.217	2	6.811	3.405	12.09	
+ milk	27	8.450	1	6.767	6.767	24.02	
+ mother	26	7.324	1	1.126	1.126	4.00	
- mother	27	8.450	-1	-1.126	1.126	4.00	0.06
+ mother	26	7.324	1	1.126	1.126	4.00	
- milk	27	10.592	-1	-3.268	3.268	11.60	0.01
+ milk	26	7.324	1	3.268	3.268	11.60	
- class	28	10.996	-2	-3.672	1.836	6.52	0.05

* Denominator of ratio is (Residual deviance/ Residual DF) of full model i.e. 7.324/26 = 0.282.

TABLE 6.7 Milk supply: body composition of pups aged 10 days
Means of litter means ± standard errors
No. of litters = 10 for each class

	controls	immigrants	eskimos
pup body weight, g	5.4 ±0.2	5.0 ±0.2	6.6*** +++ ±0.3
body fat, %	9.2 ±0.8	8.0 ±0.8	14.4*** +++ ±0.6
body water, %	71.2 ±0.8	72.7 ±0.7	67.7*** +++ ±0.7
fat free body weight, g	4.9 ±0.1	4.5 ±0.2	5.7*** +++ ±0.2

*** different from controls $P<0.001$

+++ different from immigrants $P<0.001$

TABLE 6.8 Milk supply: body composition of pups aged 10 days
Correlation of percent body fat with body weight

	Correlation coefficient	P=
Controls	0.65	0.04
Immigrants	0.69	0.03
Eskimos	0.56	0.09

10. FIGURES

LIST OF FIGURES

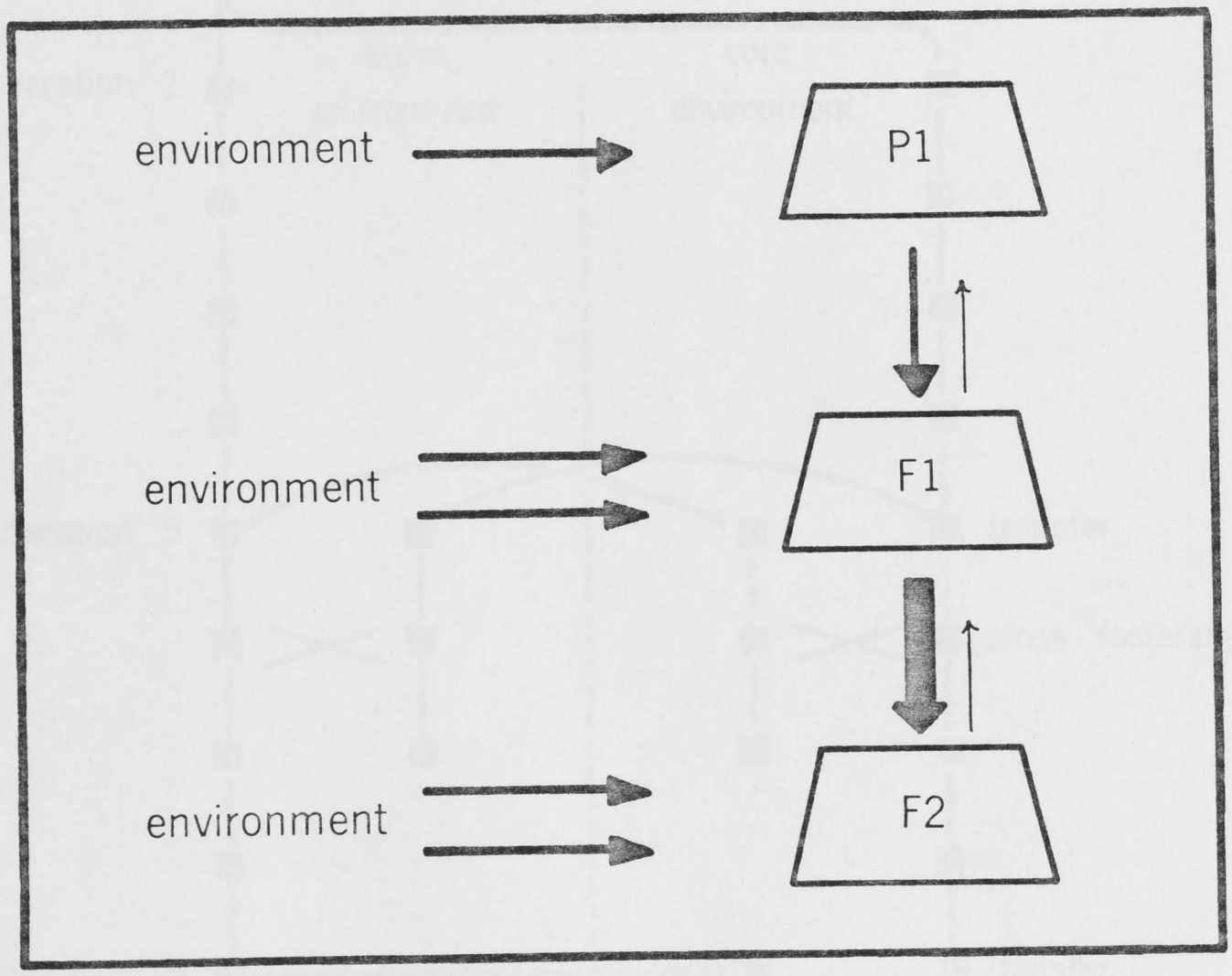

Fig. 2.1 Cummulative maternal effects. Pl is the first generation in a new environment. The environment affects Pl to produce an altered phenotype. The Fl generation is also influenced by the environment and (via maternal effects) by the altered phenotype of Pl. Similarly F2 is affected by the environment, by FI and indirectly by the influences affecting Fl. The thickening arrows indicate how these maternal effects might accumulate over several generations.

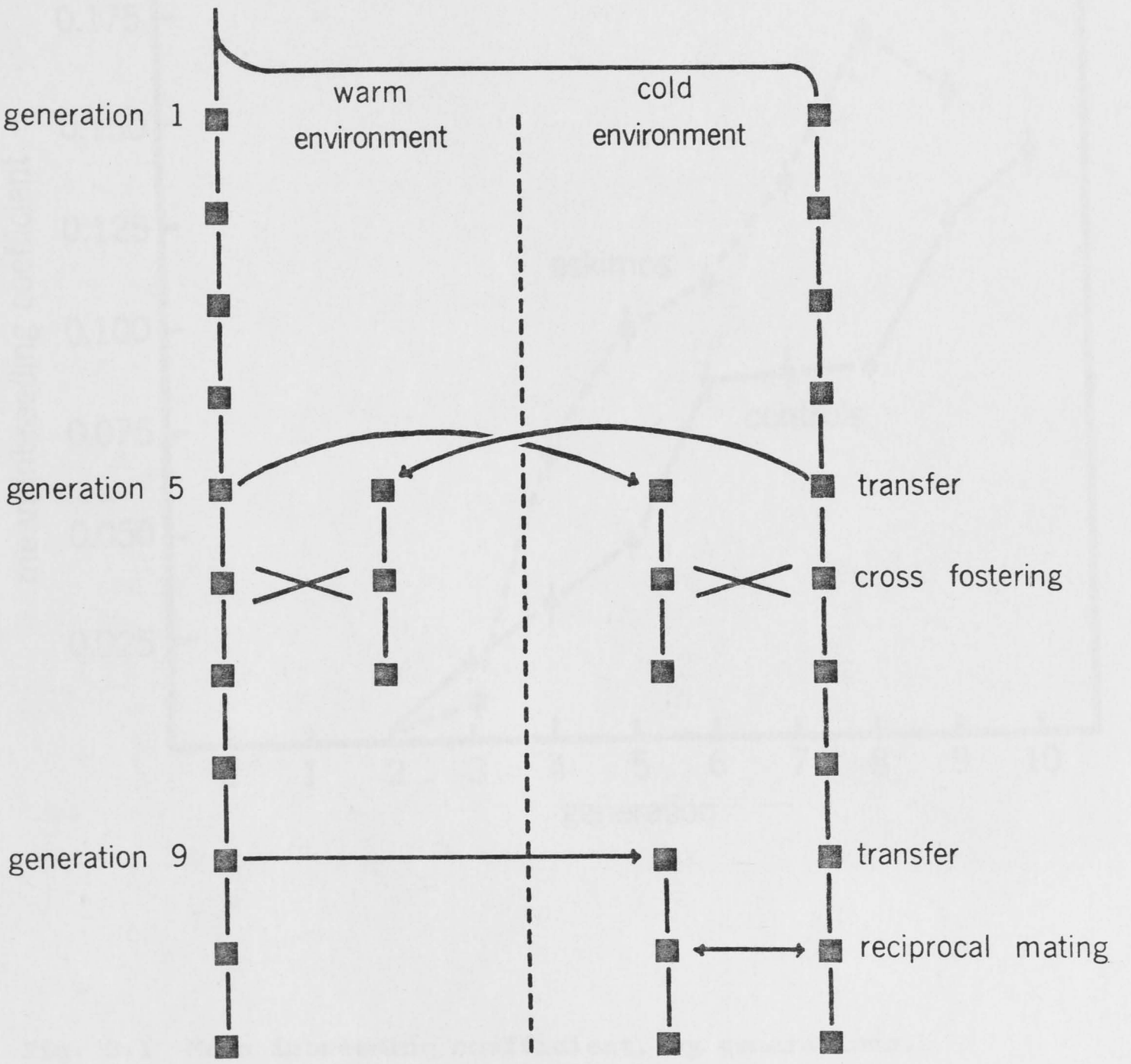

Fig. 2.2 Experimental design. The original wild trapped population is bred in the warm. Generation 1 is divided between warm and cold environments and the two populations ('controls' and eskimos) are bred concurrently. At generation 5 some pairs from each population are transferred to the opposite environment. The young of generation 6 are fostered between transfers and indigenes in each environment. At generation 9 some control pairs are transferred to the cold environment. Their offspring are reciprocally mated with the eskimos.

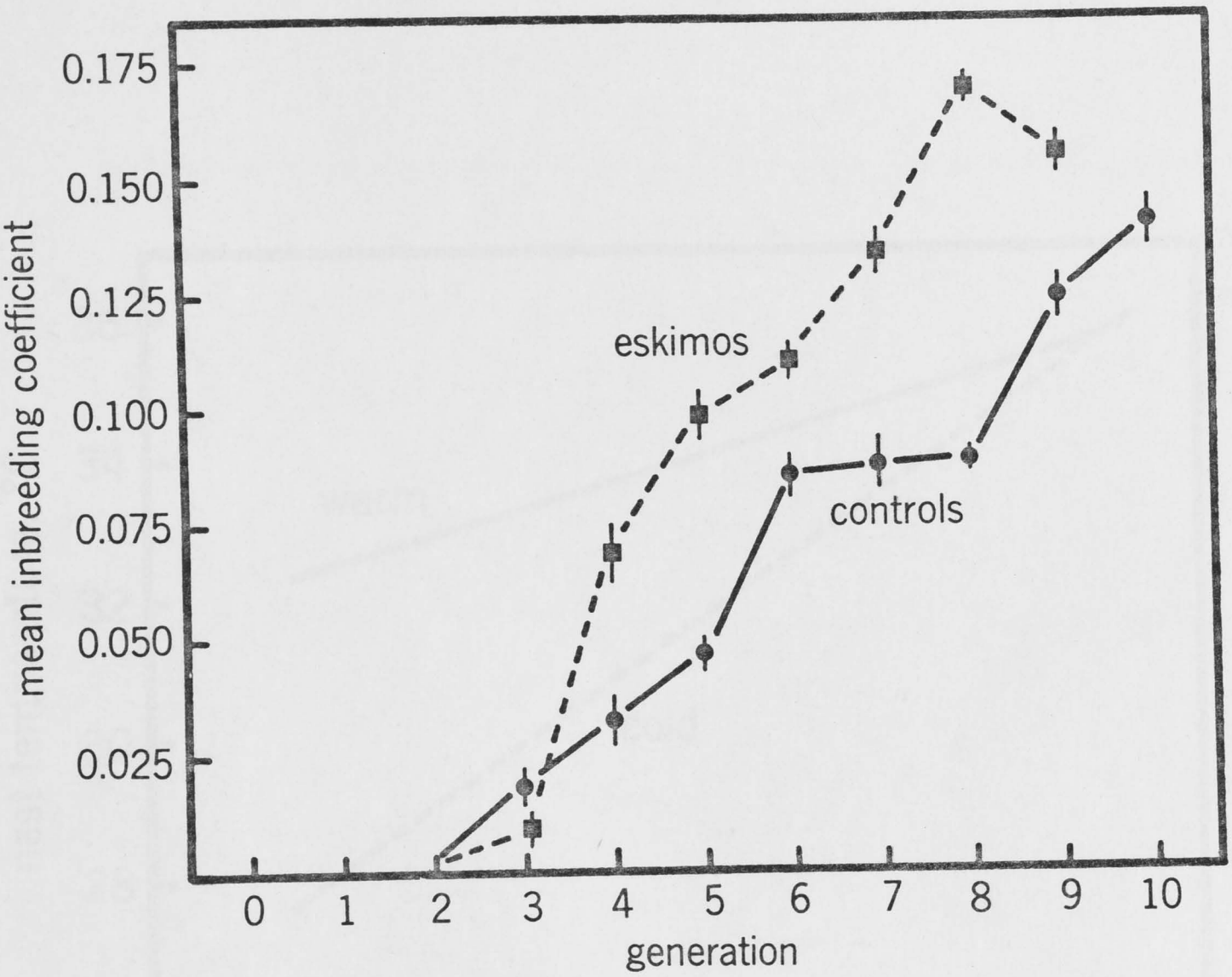

Fig. 3.1 Mean inbreeding coefficient, by generations.
Vertical bars represent standard errors.

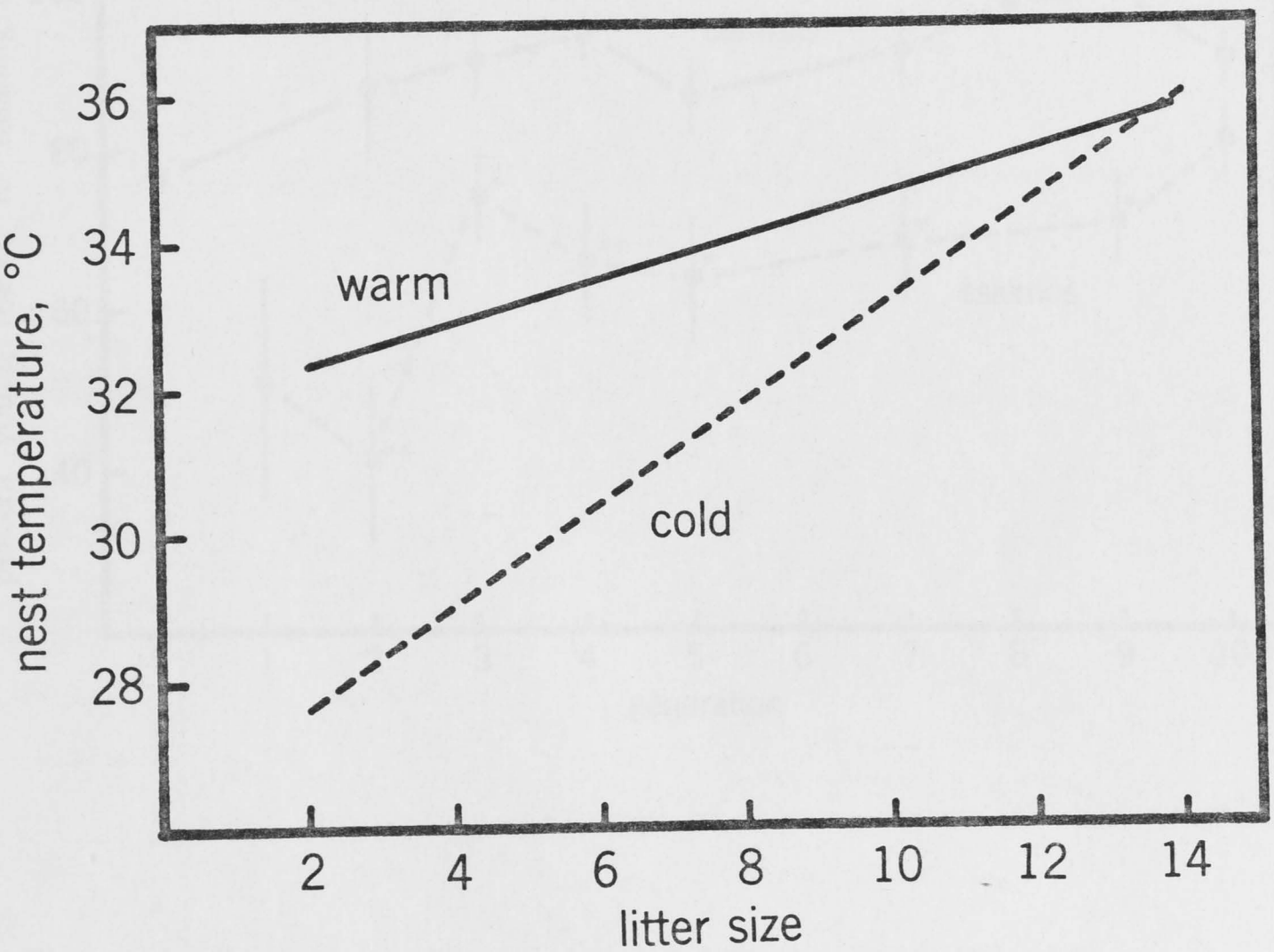

Fig. 3.2 Regression of mid-young nest temperature on litter size. Warm environment r = 0.50 ($P<0.05$); cold environment r = 0.61 ($P<0.01$).

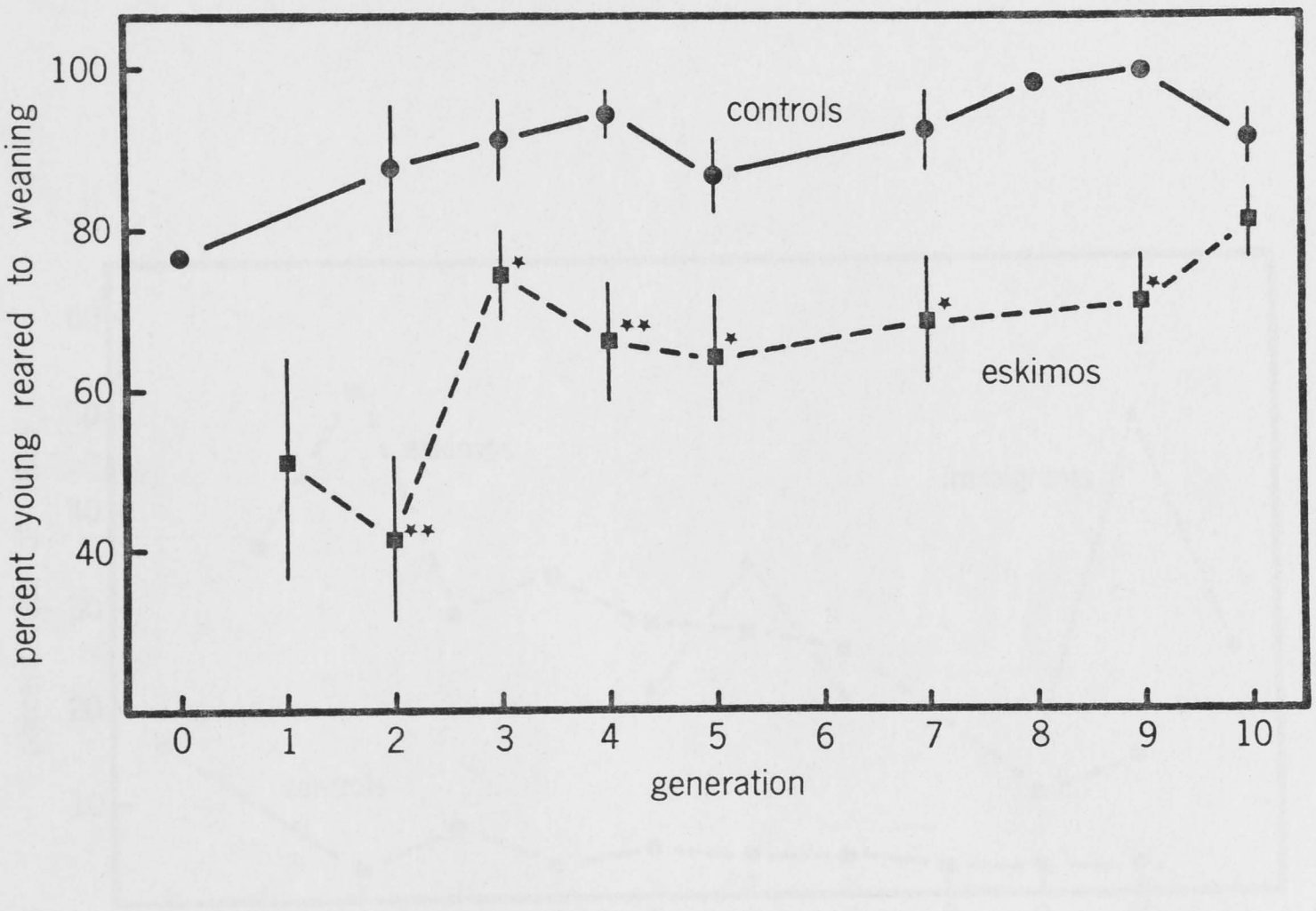

Fig. 4.1 Mean young reared to weaning per pair (%), by generations. Vertical bars represent standard errors.
* $P<0.05$
** $P<0.01$

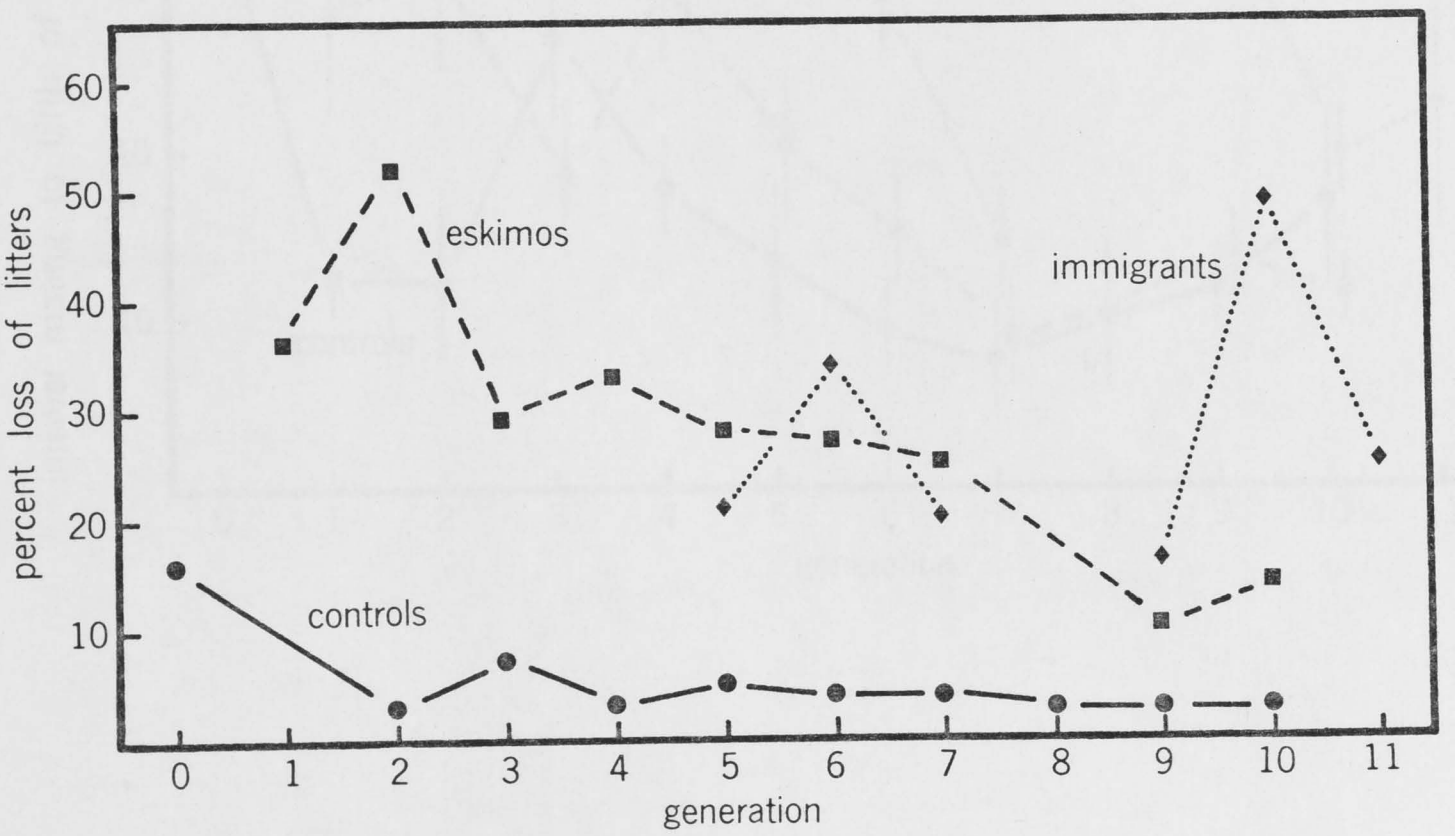

Fig. 4.2 Loss of whole litters per class (%), by generations. Dotted lines: controls transferred to the cold (immigrants).

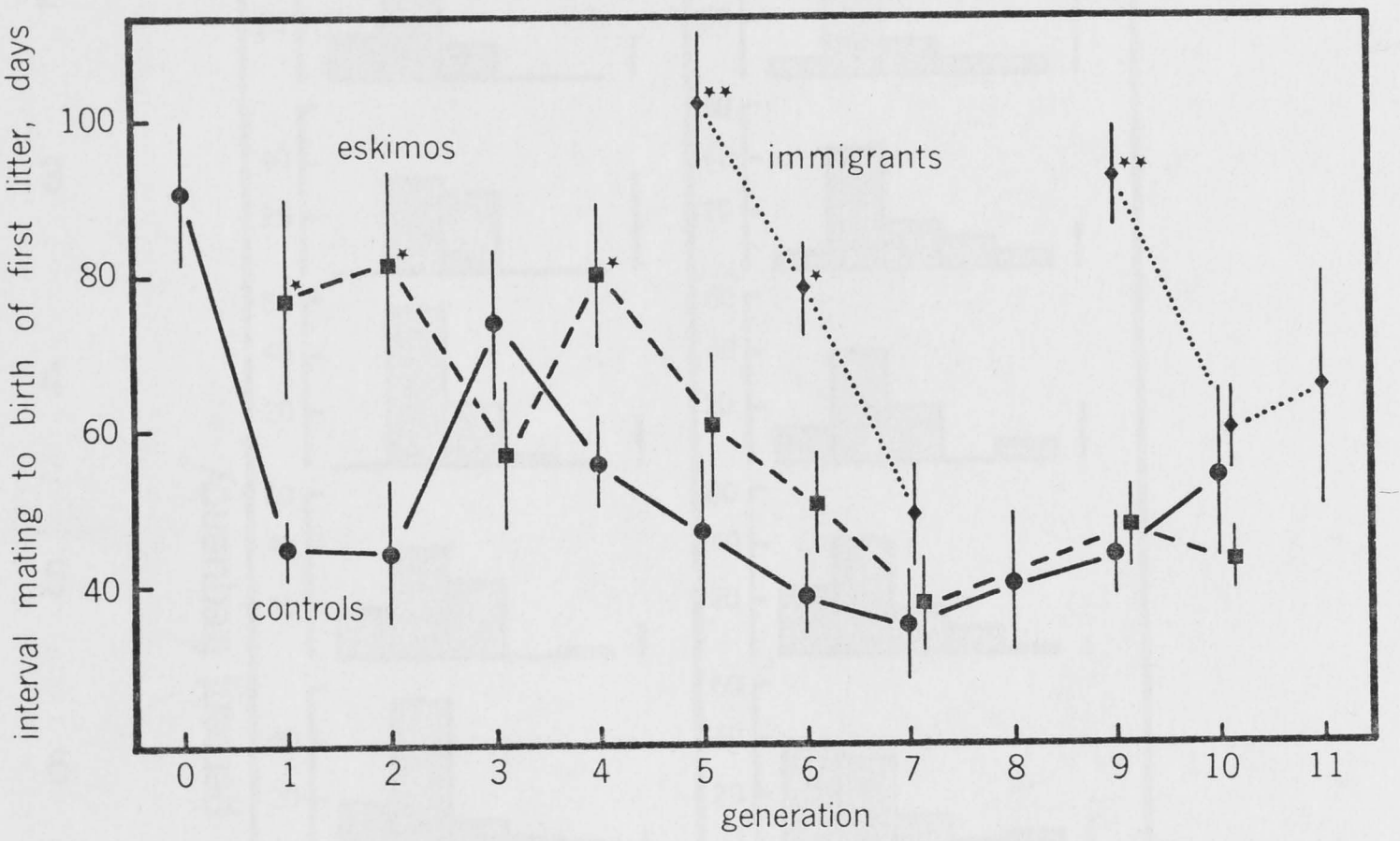

Fig. 4.3 Mean duration of intervals between mating and birth of first litter, by generations.
Dotted lines: controls transferred to the cold (immigrants). Vertical bars represent standard errors.

* Different from controls $P<0.05$

** Different from controls $P<0.01$

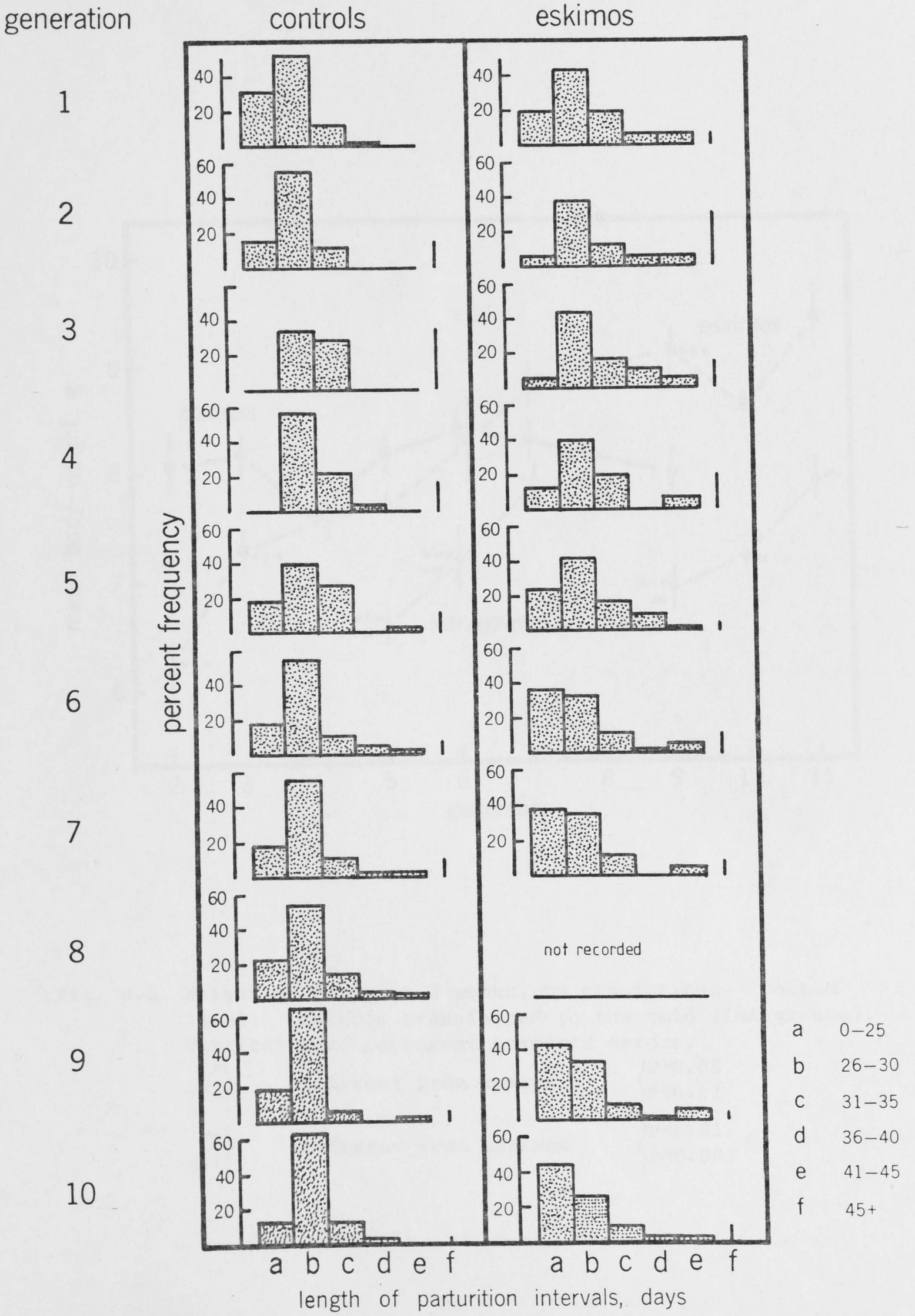

Fig. 4.4 Change over generations in distribution of parturition intervals.

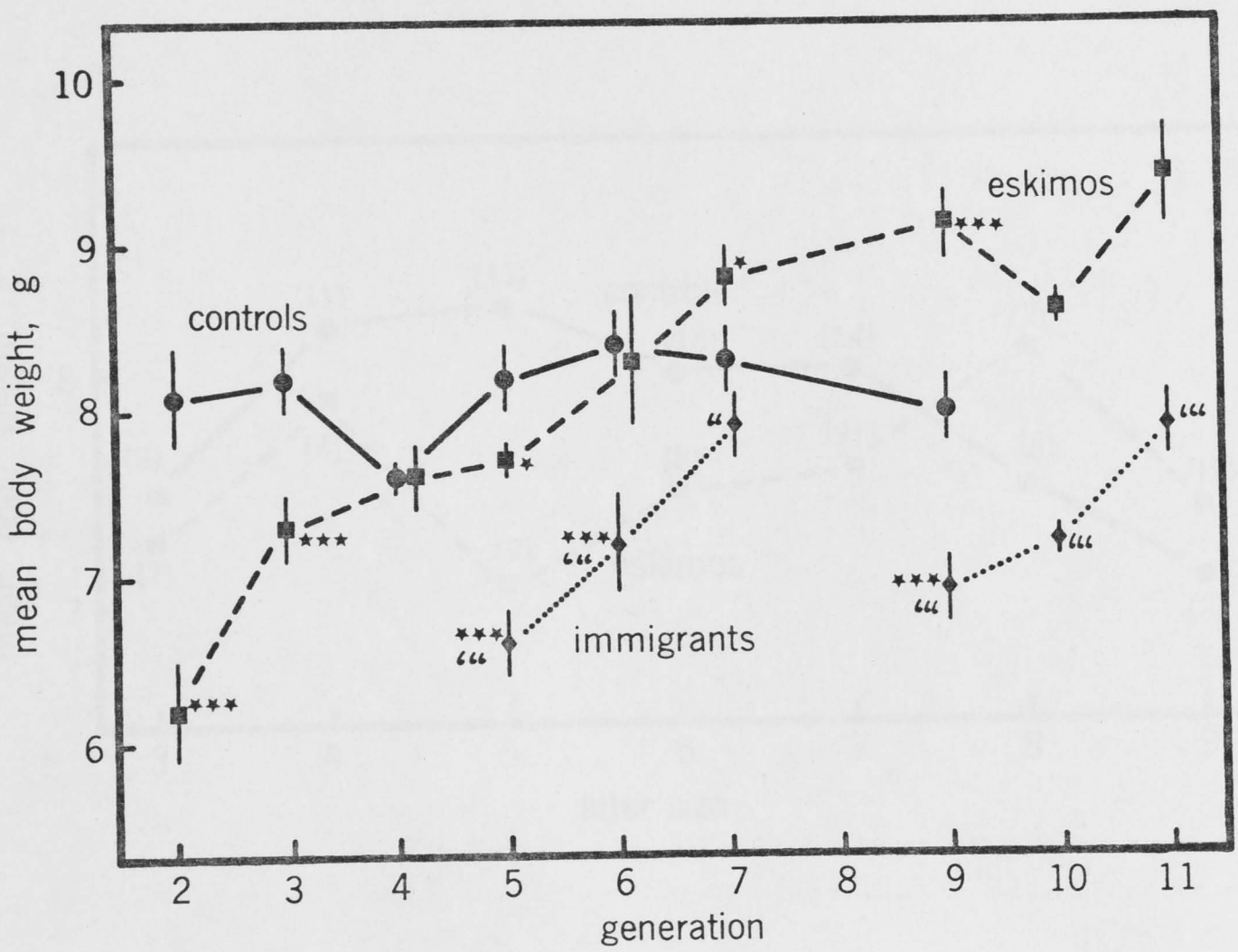

Fig. 4.5 Weight of young at 3 weeks, by generations. Dotted lines: controls transferred to the cold (immigrants). Vertical bars represent standard errors.

* , *** different from controls: $P < 0.05$, $P < 0.01$

“ , “‘ different from eskimos: $P < 0.01$, $P < 0.001$

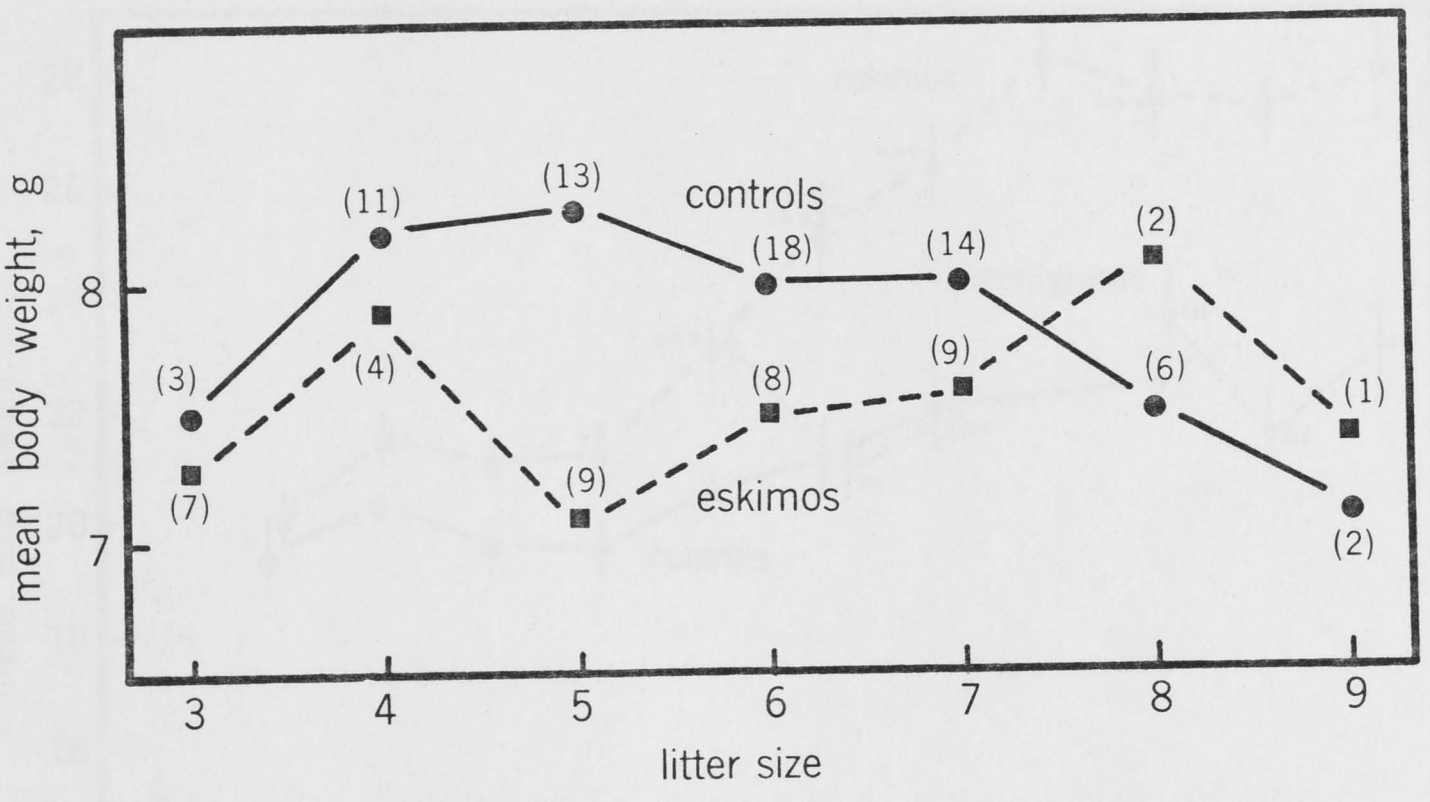

Fig. 4.6 Litter size and individual weight of young at 3 weeks. Continuous line: controls of generations 2-5. Broken line: eskimos of generations 3-5. Number of litters in parentheses.

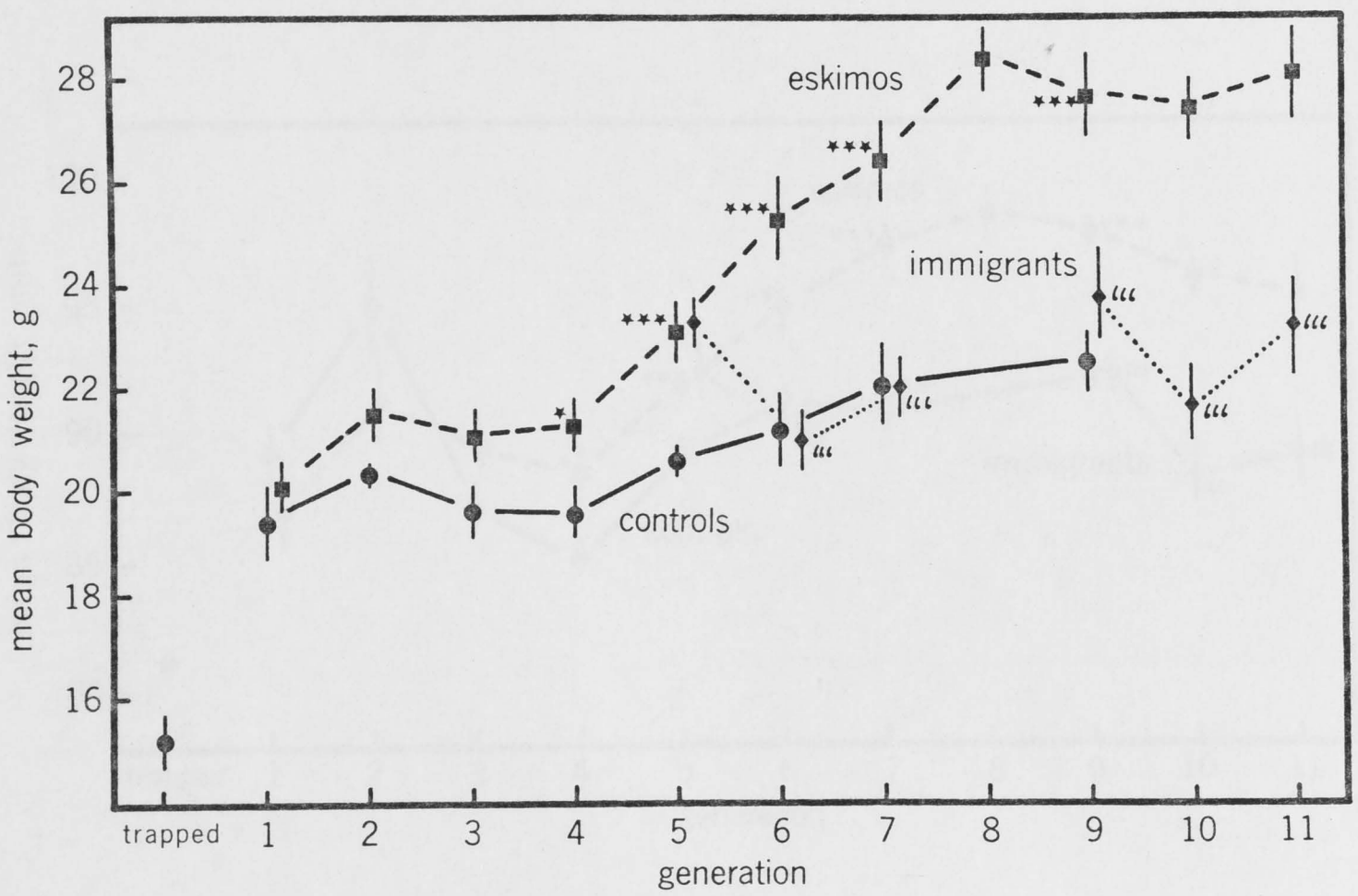

Fig. 4.7 Body weights of males aged 30 weeks or older, by generations. Dotted lines: controls transferred to the cold (immigrants). Vertical lines represent standard errors.

* different from controls P≤0.05
*** different from controls P≤0.001
‘‘‘ different from eskimos P≤0.001

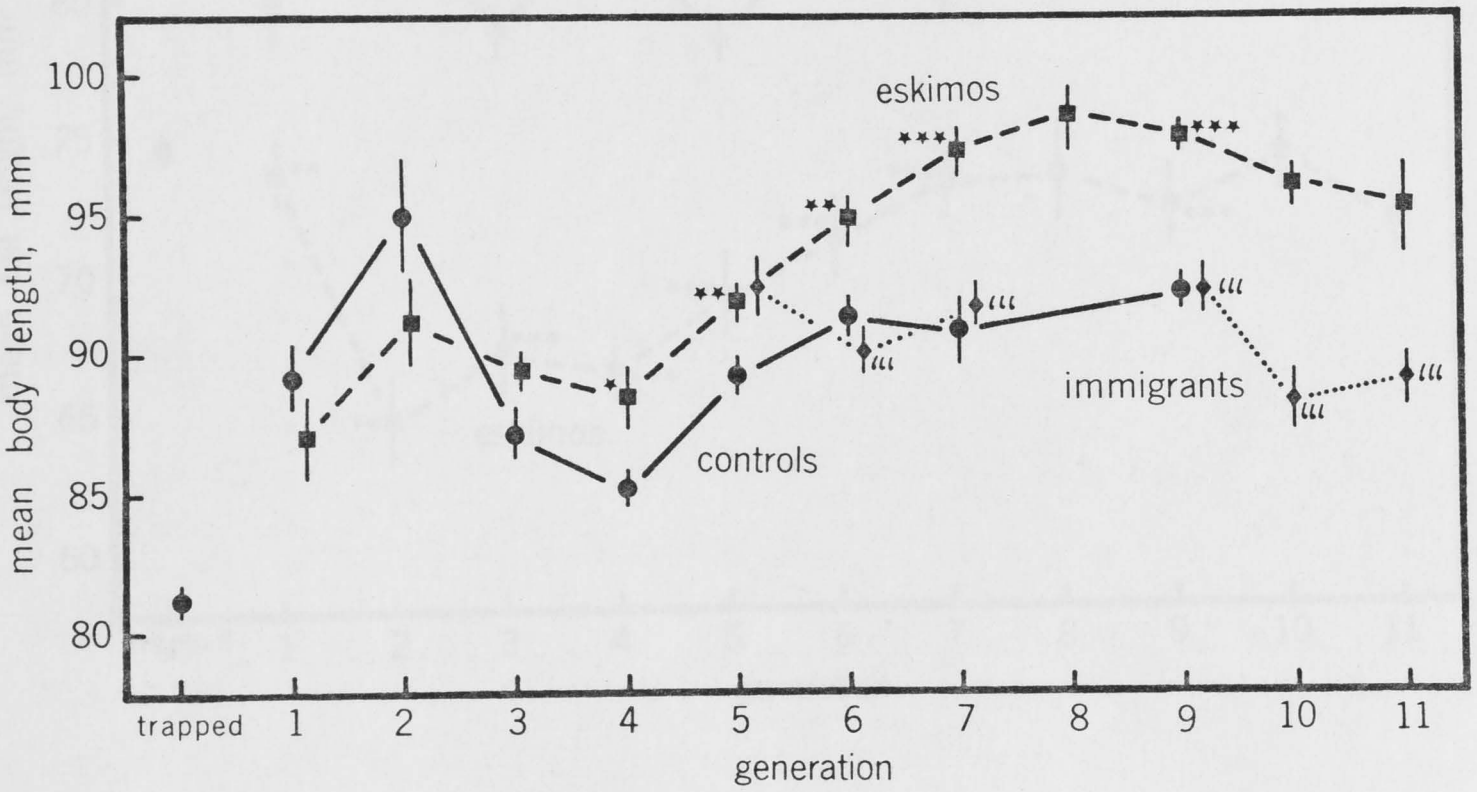

Fig. 4.8 Body lengths of males aged 30 weeks or older, by generations. Dotted lines: controls transferred to the cold (immigrants). Vertical bars represent standard errors.

*, **, *** different from controls: P<0.05, P<0.01, P<0.001

‘‘‘ different from eskimos: P<0.001

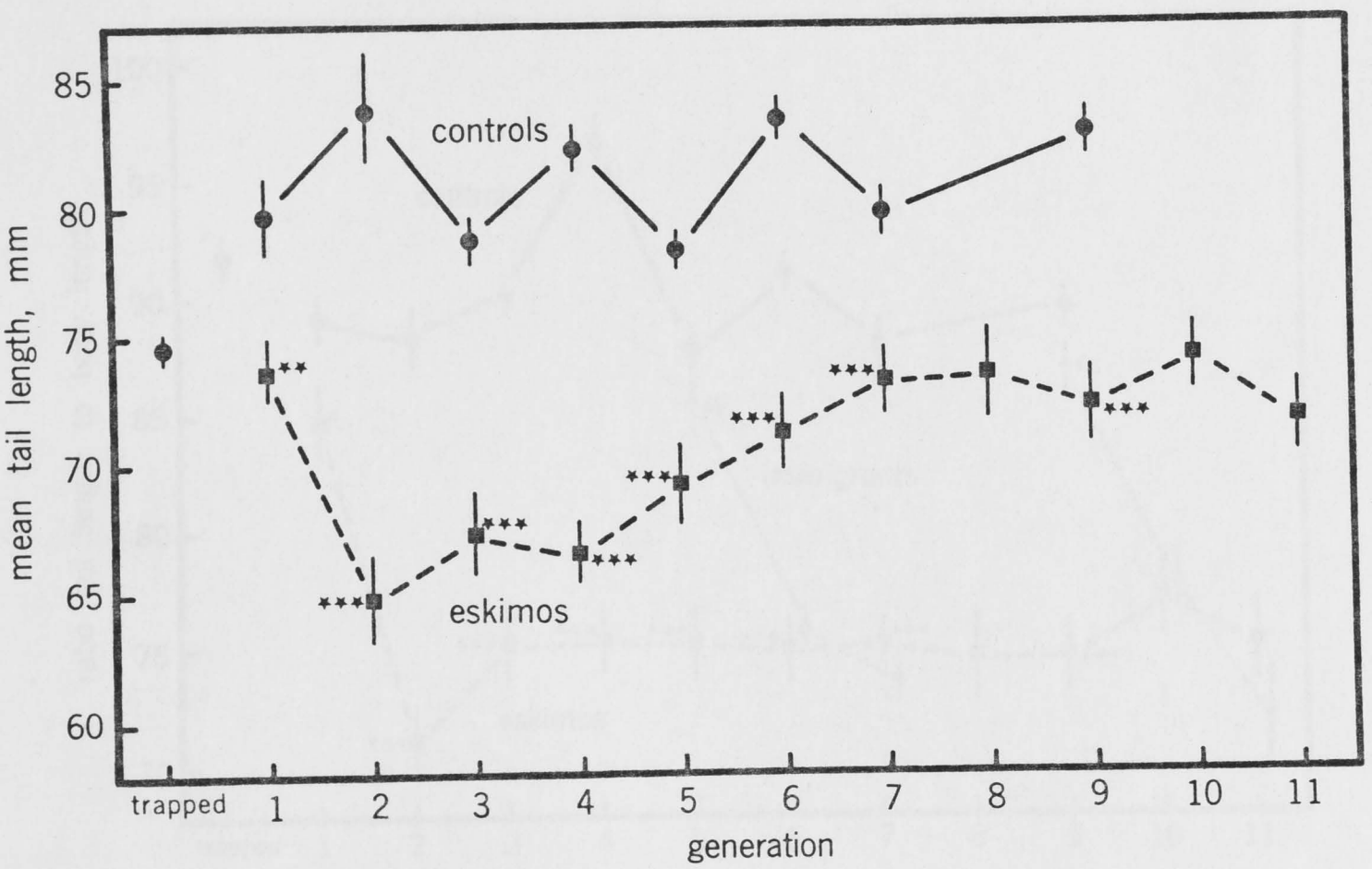

Fig. 4.9 Tail lengths of males aged 30 weeks or older, by generation. Vertical lines represent standard errors.
** $P<0.01$
*** $P<0.001$

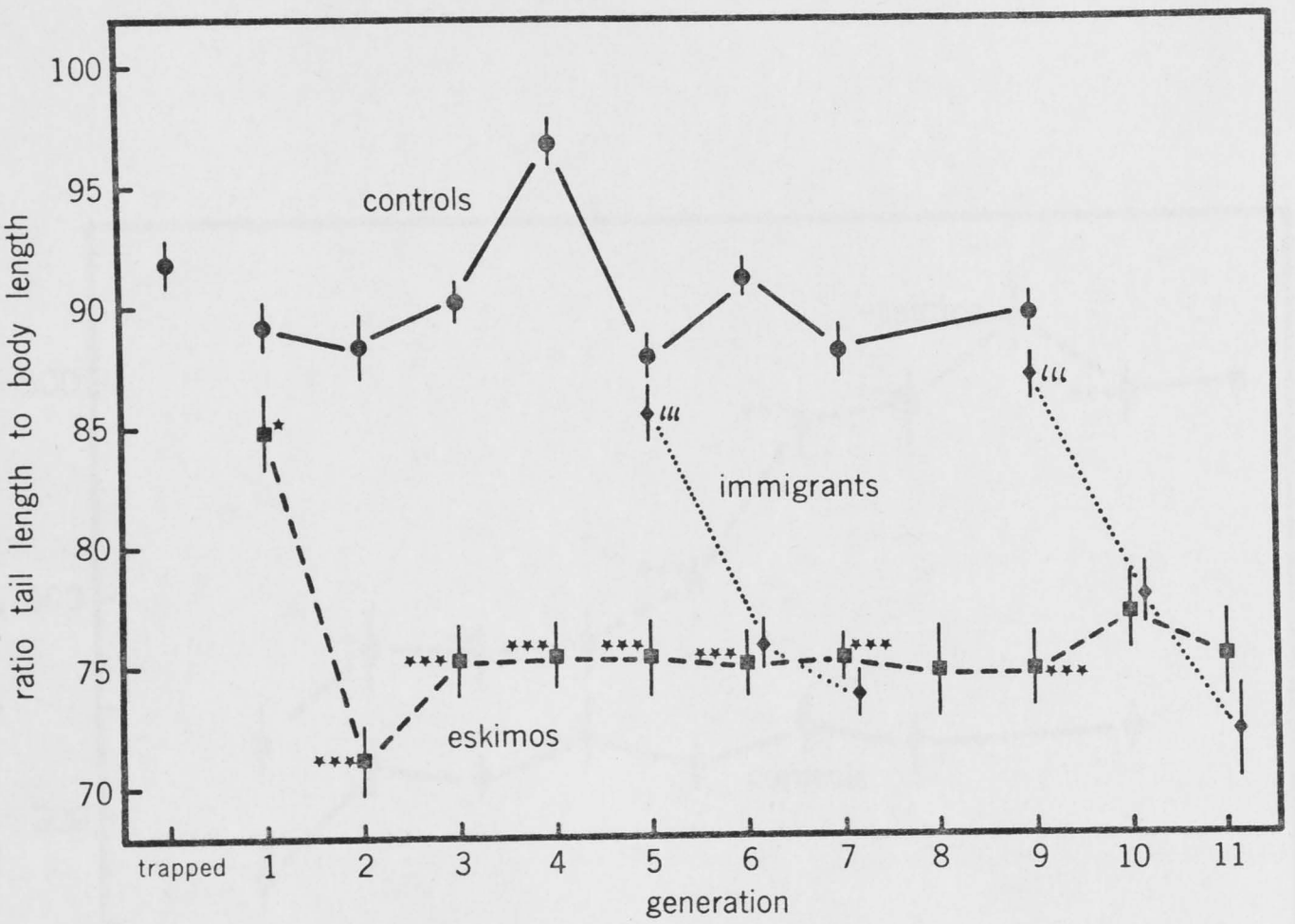

Fig. 4.10 Ratios of tail to body lengths of males aged 30 weeks or older, by generation. Dotted lines: controls transferred to the cold (immigrants). Vertical lines represent standard errors.

***	different from controls	P<0.001
‘‘‘	different from eskimos	P<0.001

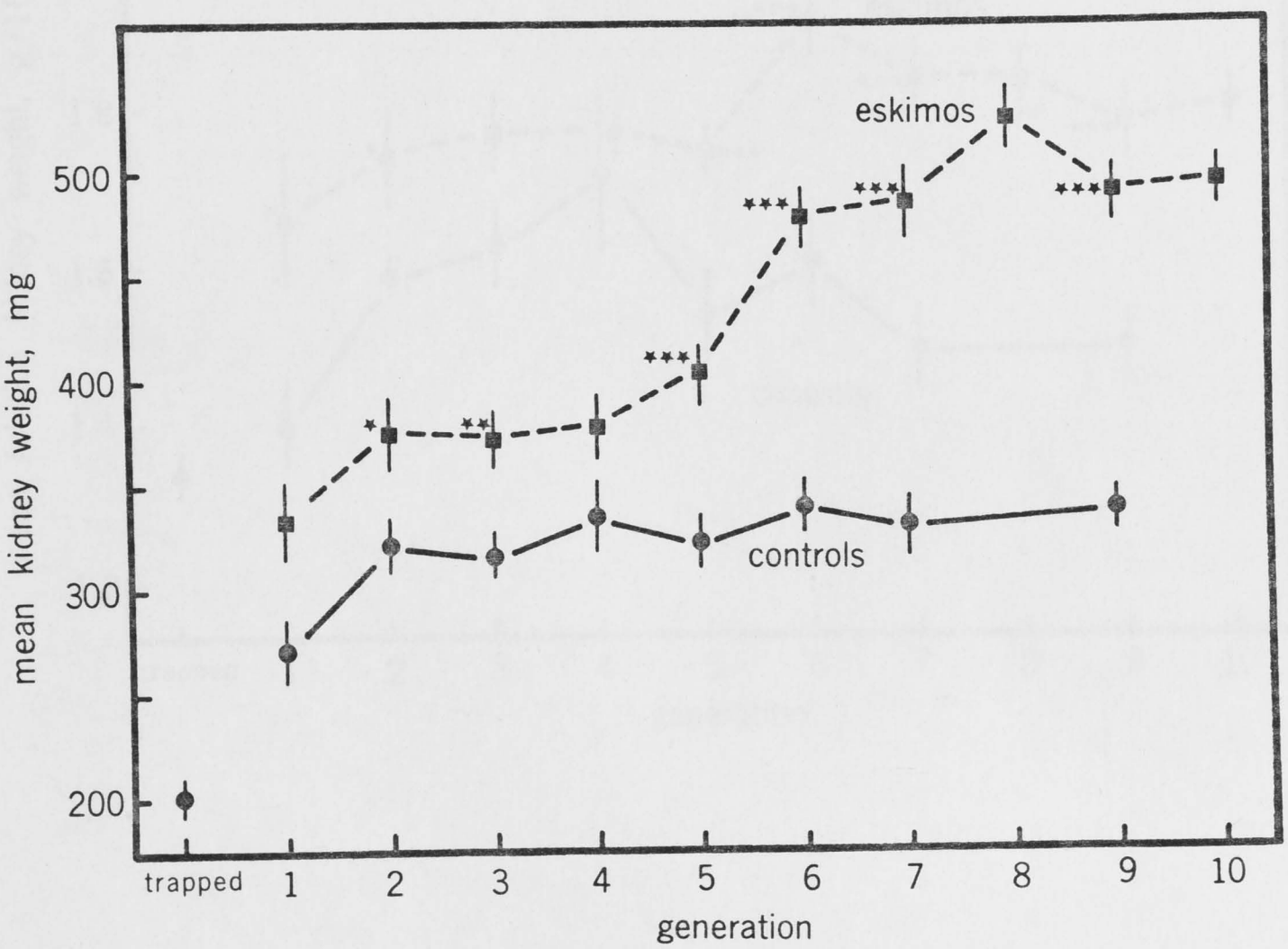

Fig. 4.11 Kidney weights of males aged 30 weeks or older, by generation. Vertical lines represent standard errors.

* $P<0.05$

** $P<0.01$

*** $P<0.001$

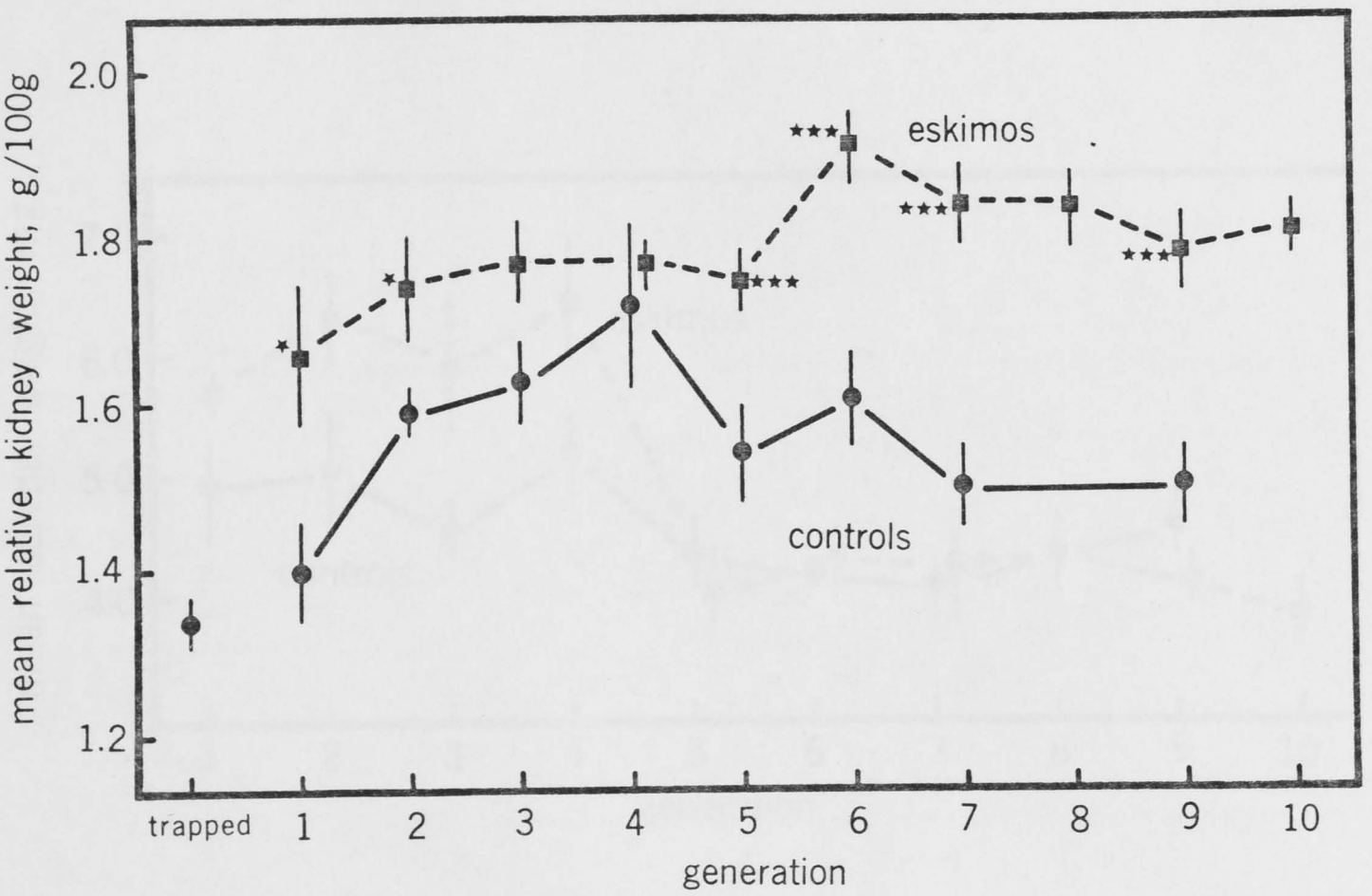

Fig. 4.12 Relative kidney weights of males aged 30 weeks or older. Vertical lines represent standard errors.
* $P<0.05$
*** $P<0.001$

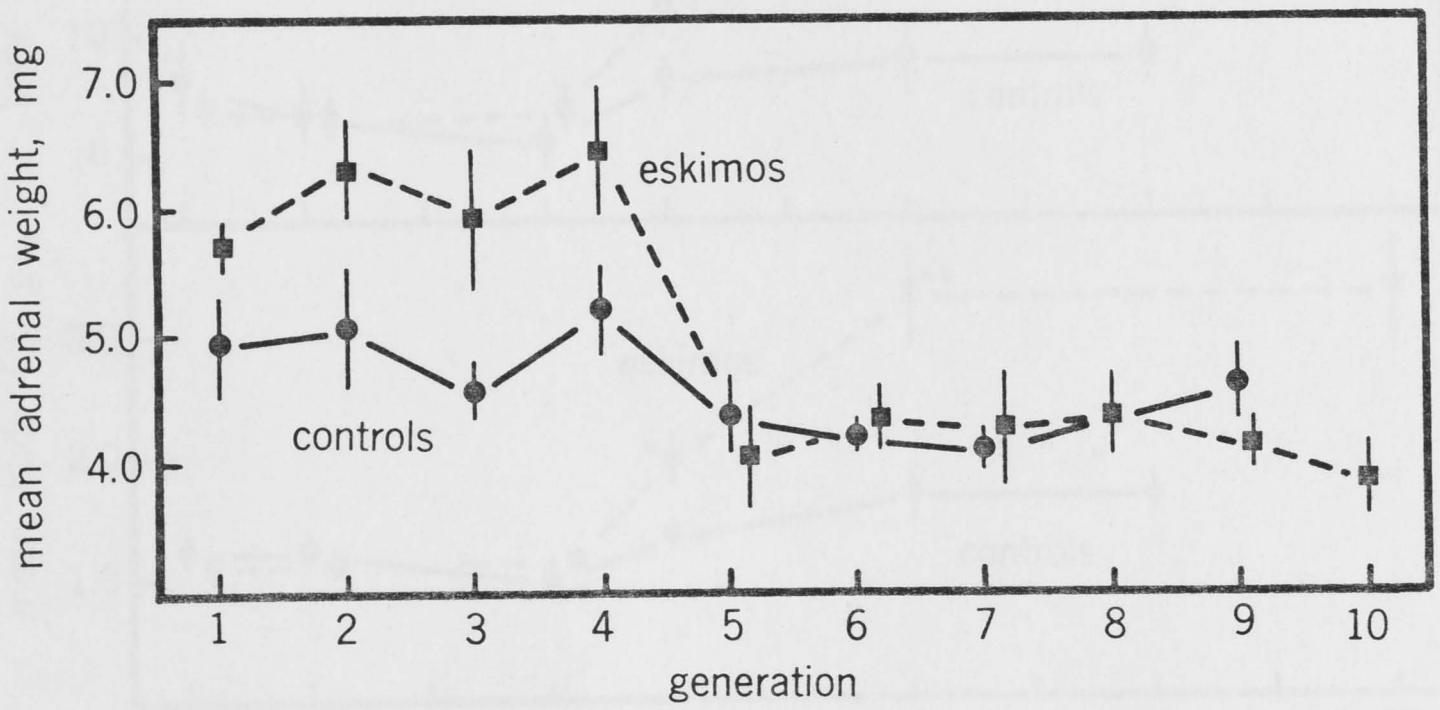

Fig. 4.13 Adrenal weights of males aged 30 weeks or older, by generations. Vertical lines represent standard errors.

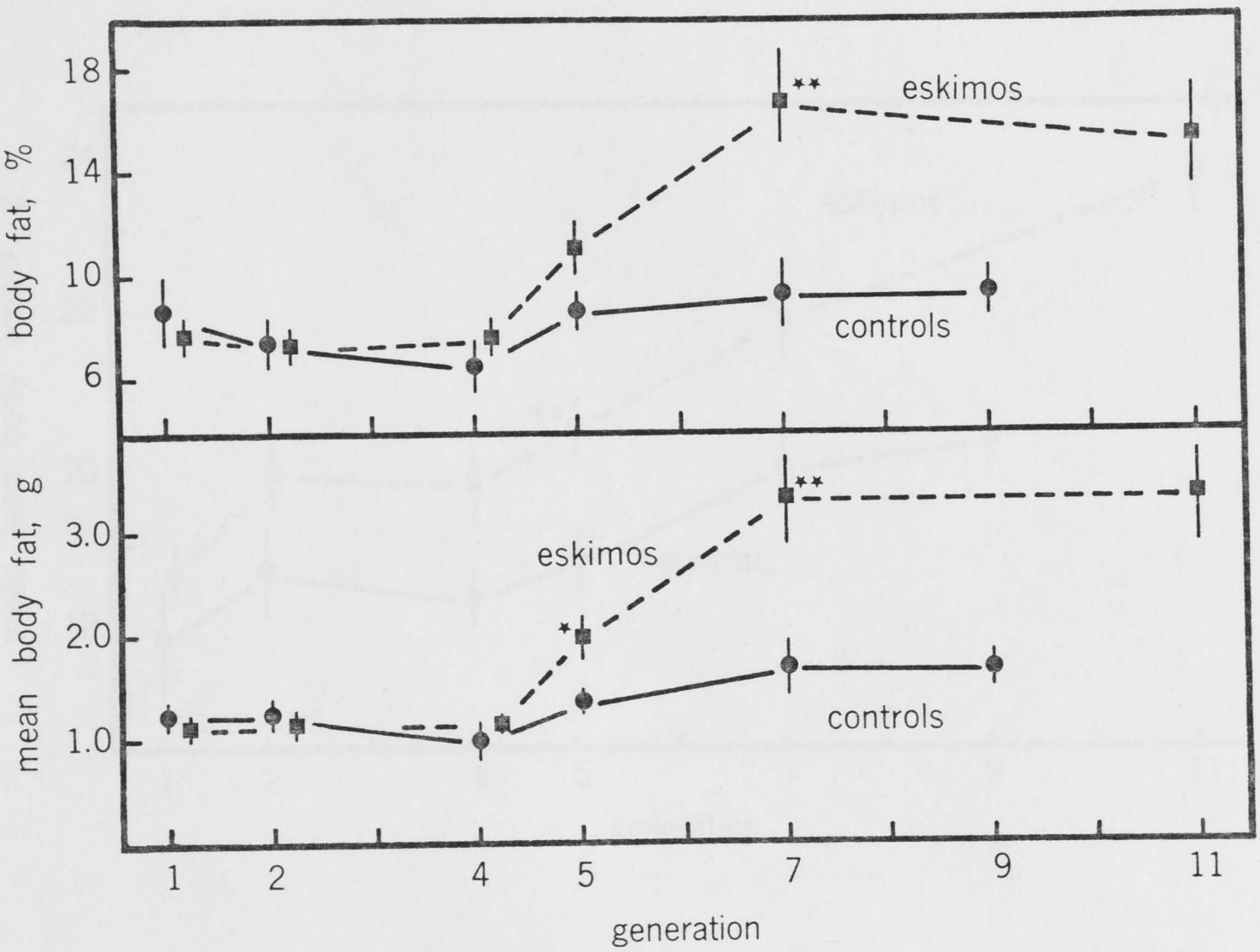

Fig. 4.14 Absolute and relative weights of body fat of males aged 30 weeks or older, by generations. Vertical lines represent standard errors.

* $P < 0.05$

** $P < 0.01$

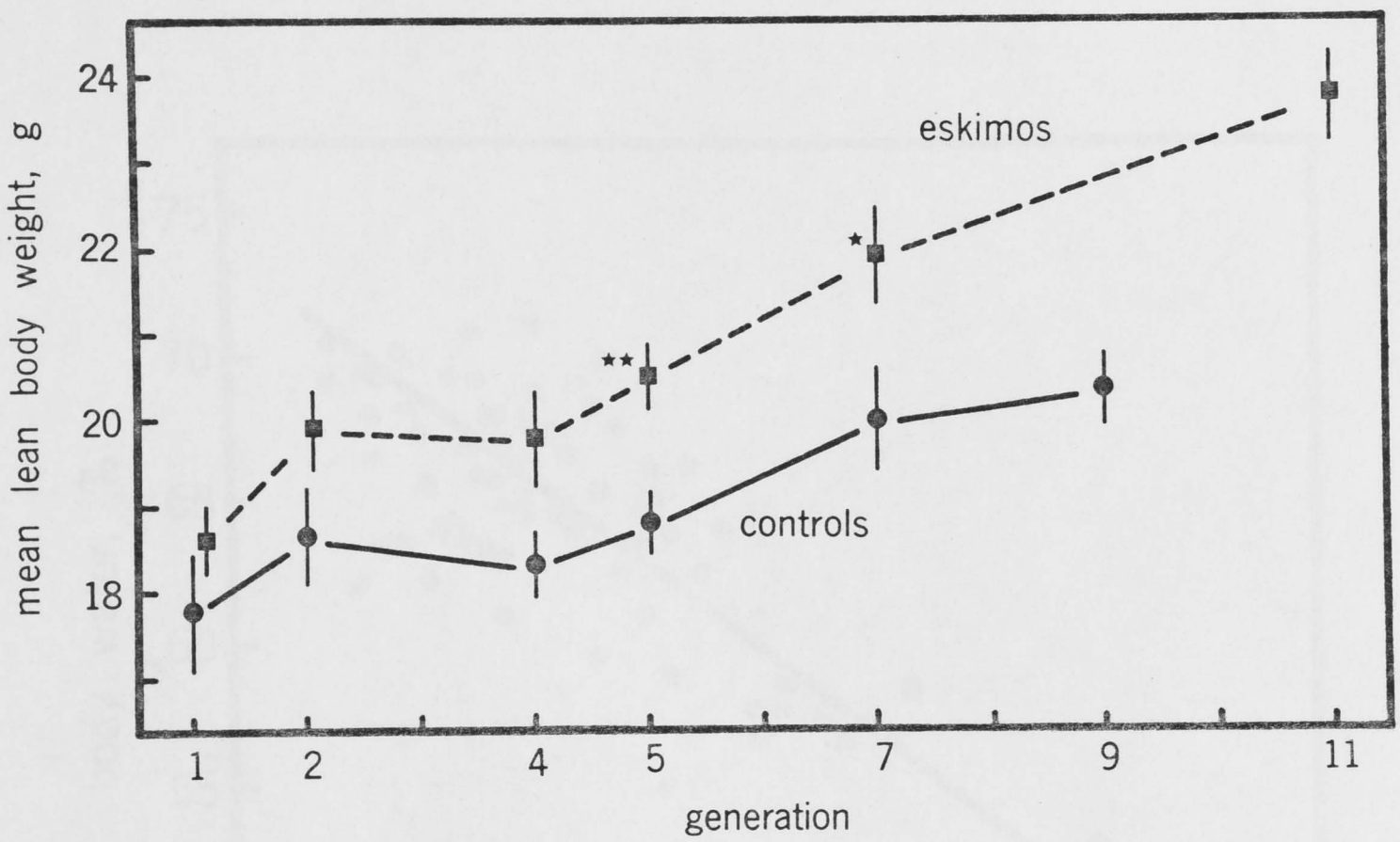

Fig. 4.15 Lean body weights of males aged 30 weeks or older, by generations. Vertical lines represent standard errors.

* $P < 0.05$

** $P < 0.01$

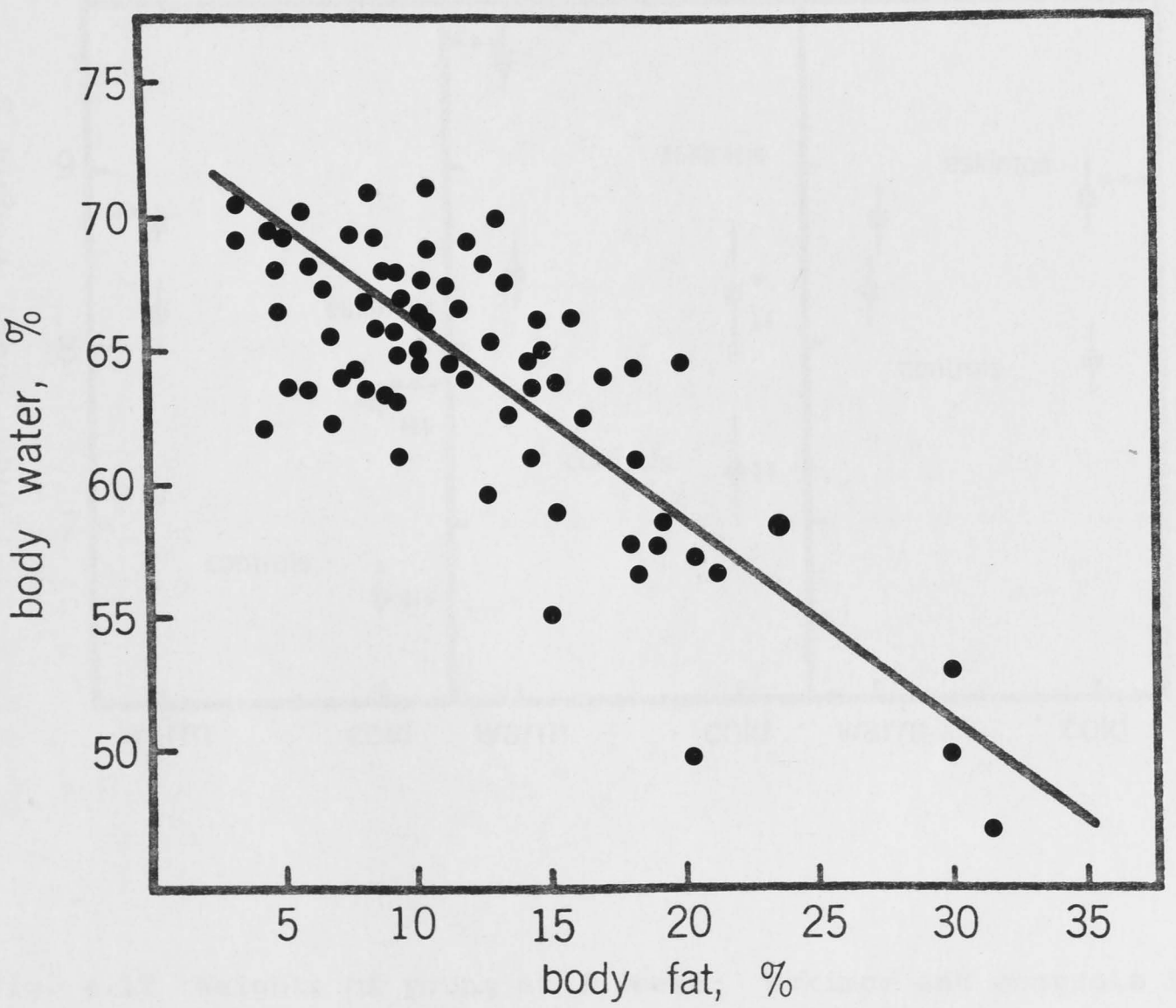

Fig. 4.16 Correlation of body water with body fat: all classes pooled. $r = -0.81$; $P < 0.001$.

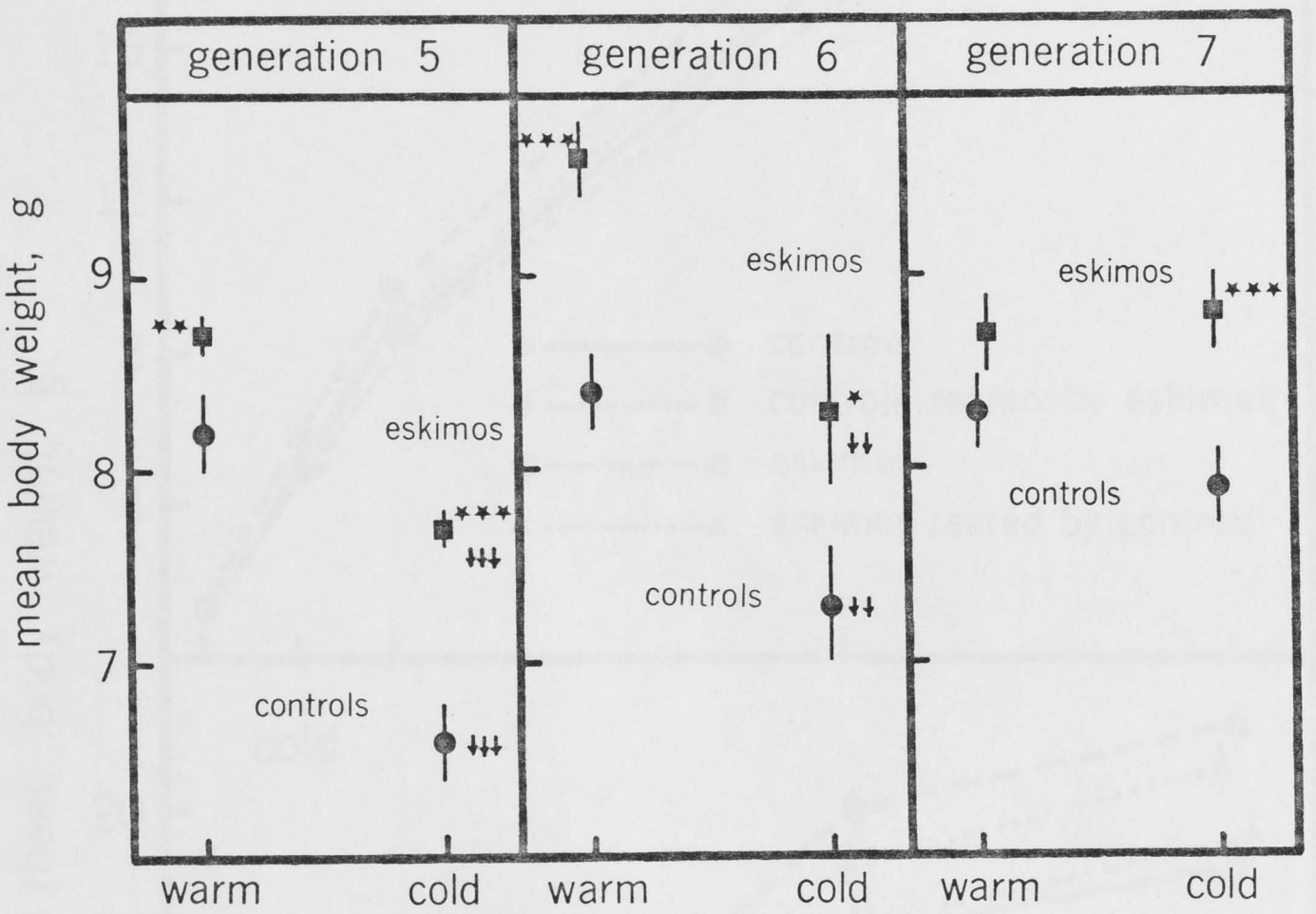

Fig. 4.17 Weights of young at 3 weeks: eskimos and controls in both environments. ■ eskimos; ● controls. Vertical lines represent standard errors.

*	different from controls	P<0.05
**		P<0.01
***		P<0.001
↓↓	different from same mouse type in the warm	P<0.01
↓↓↓		P<0.001

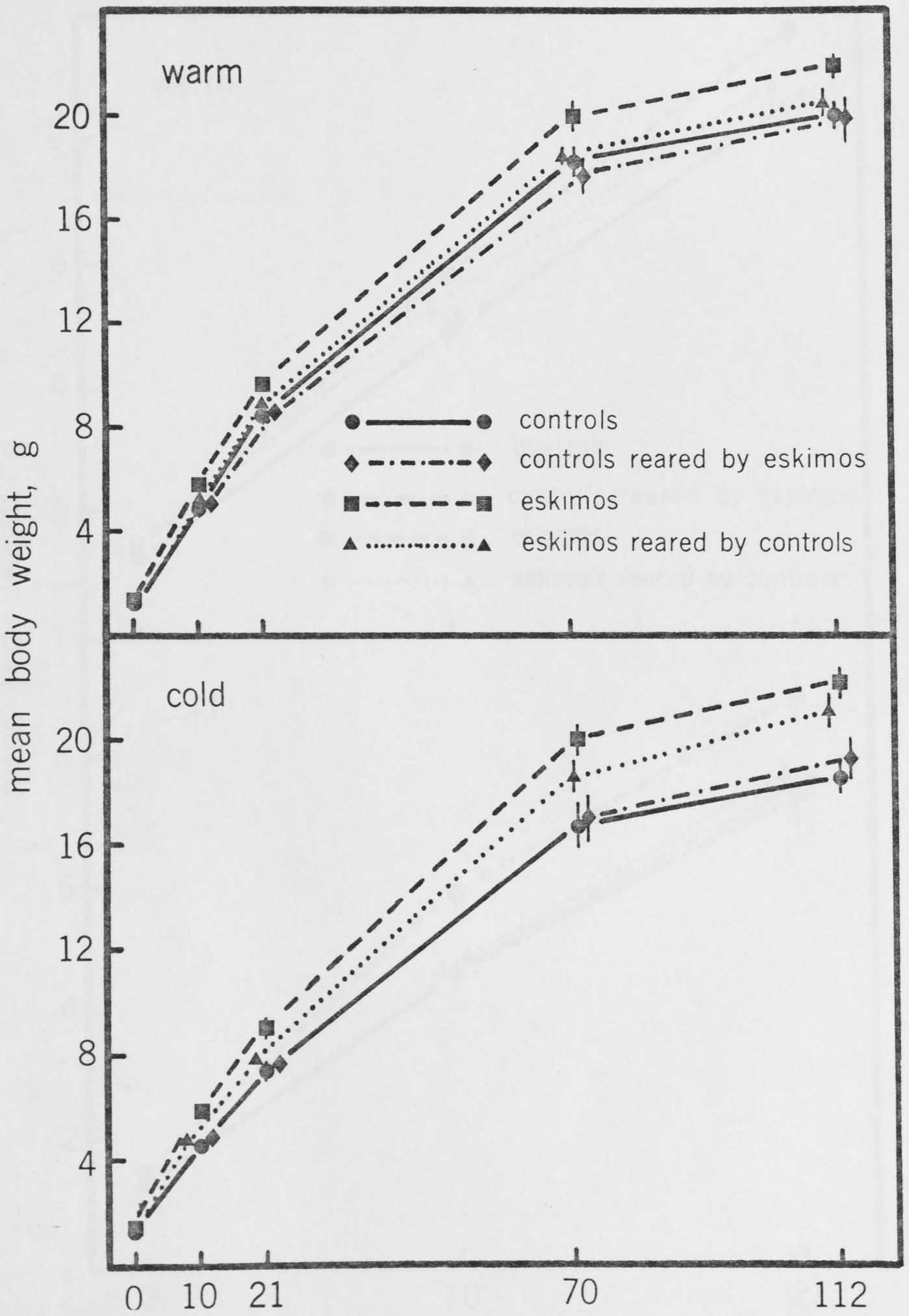

Fig. 5.1 Growth of fostered mice. Vertical bars represent standard errors.

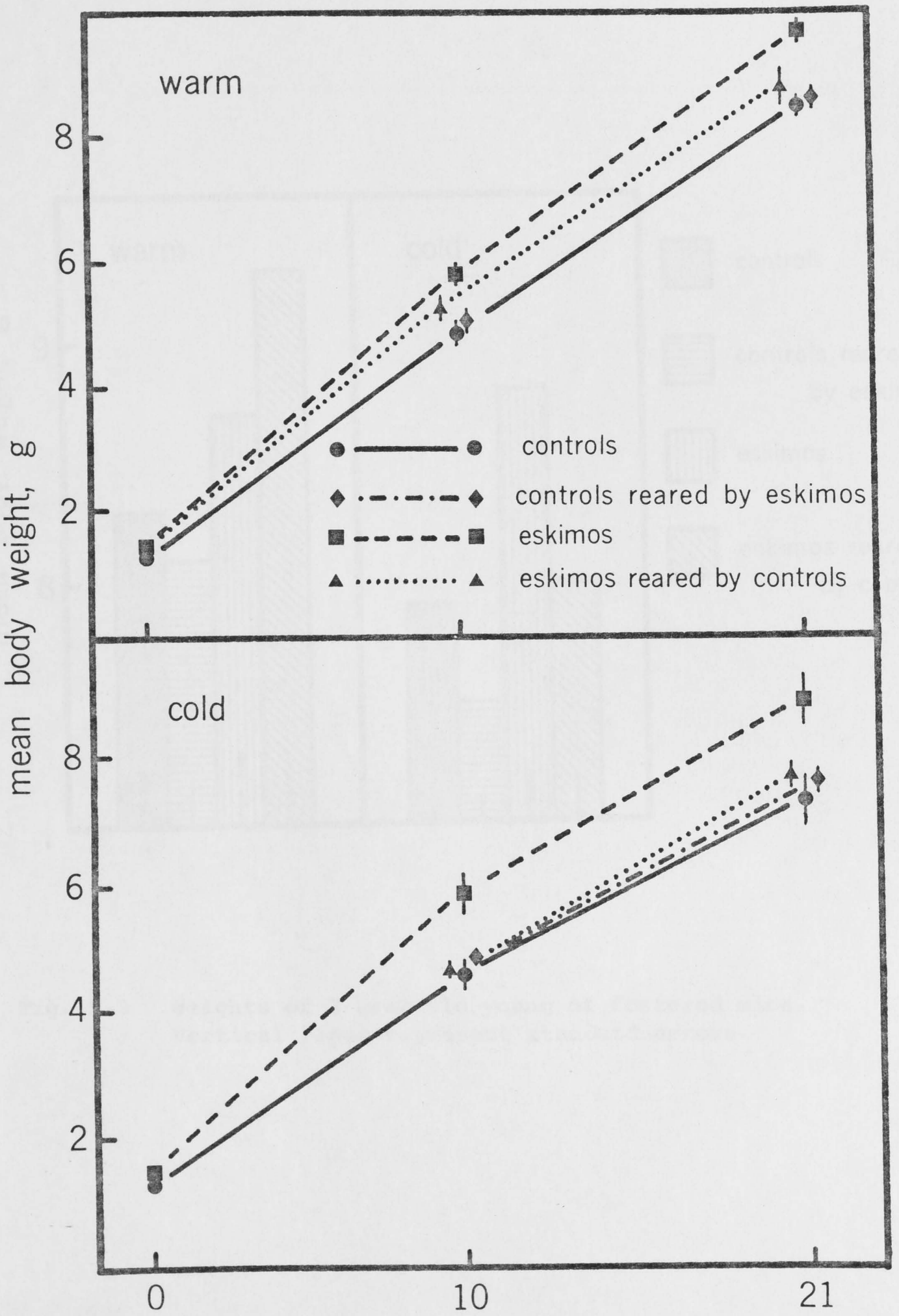

Fig. 5.2 Growth of fostered mice to 21 days. Vertical bars represent standard errors.

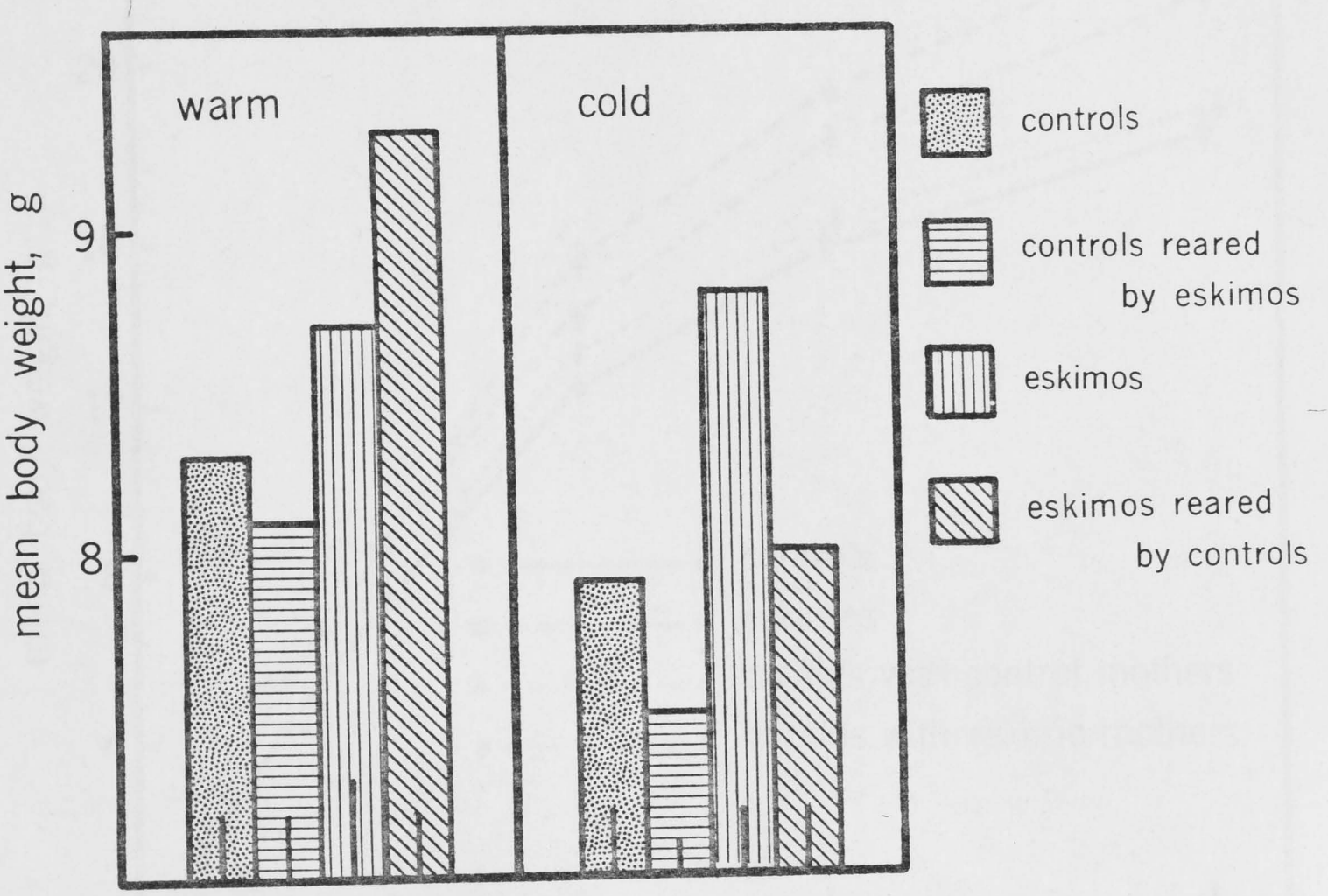

Fig. 5.3 Weights of 3 week old young of fostered mice. Vertical lines represent standard errors.

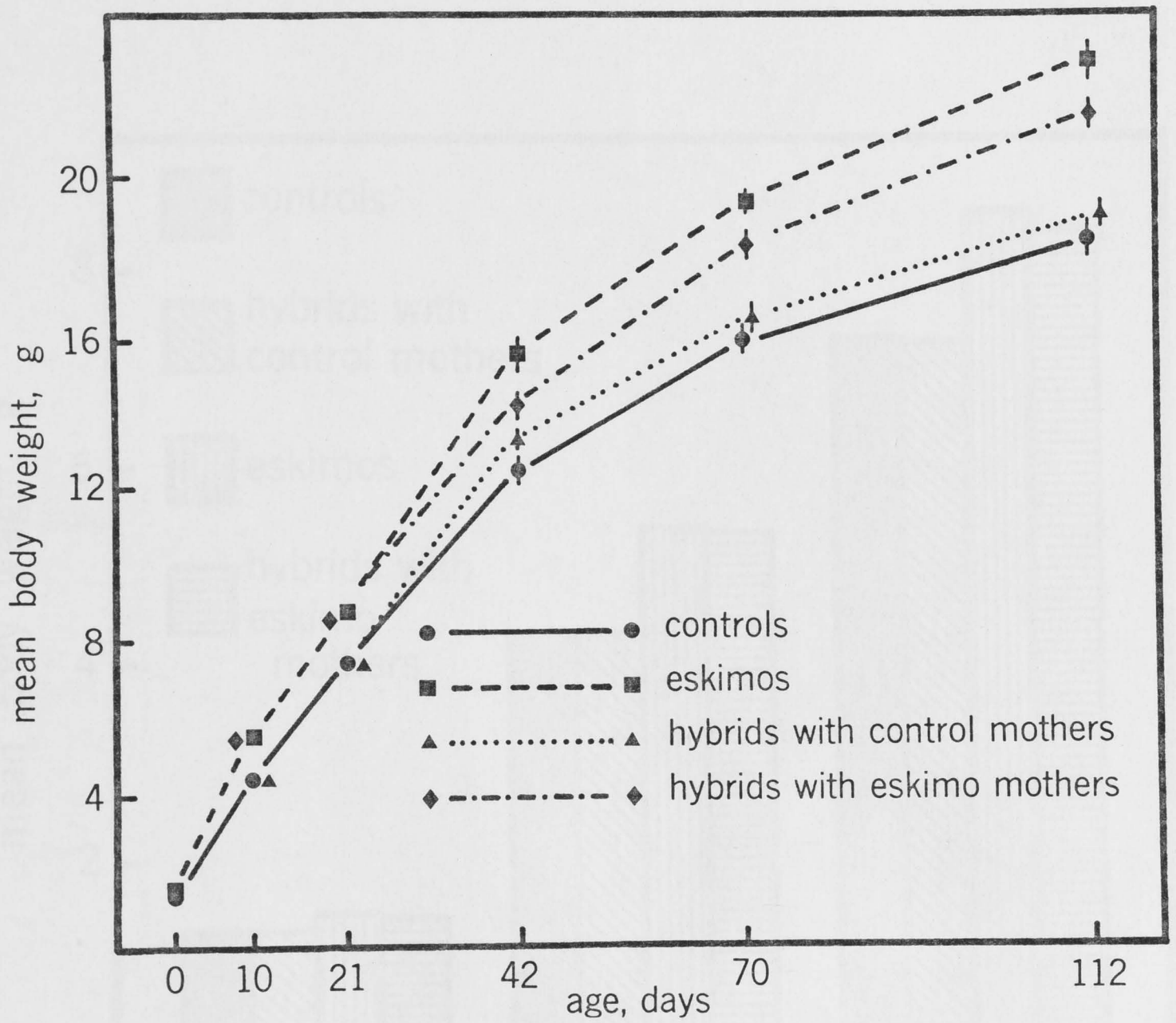

Fig. 5.4 Growth of hybrid mice compared with that of controls and eskimos. Vertical lines represent standard errors.

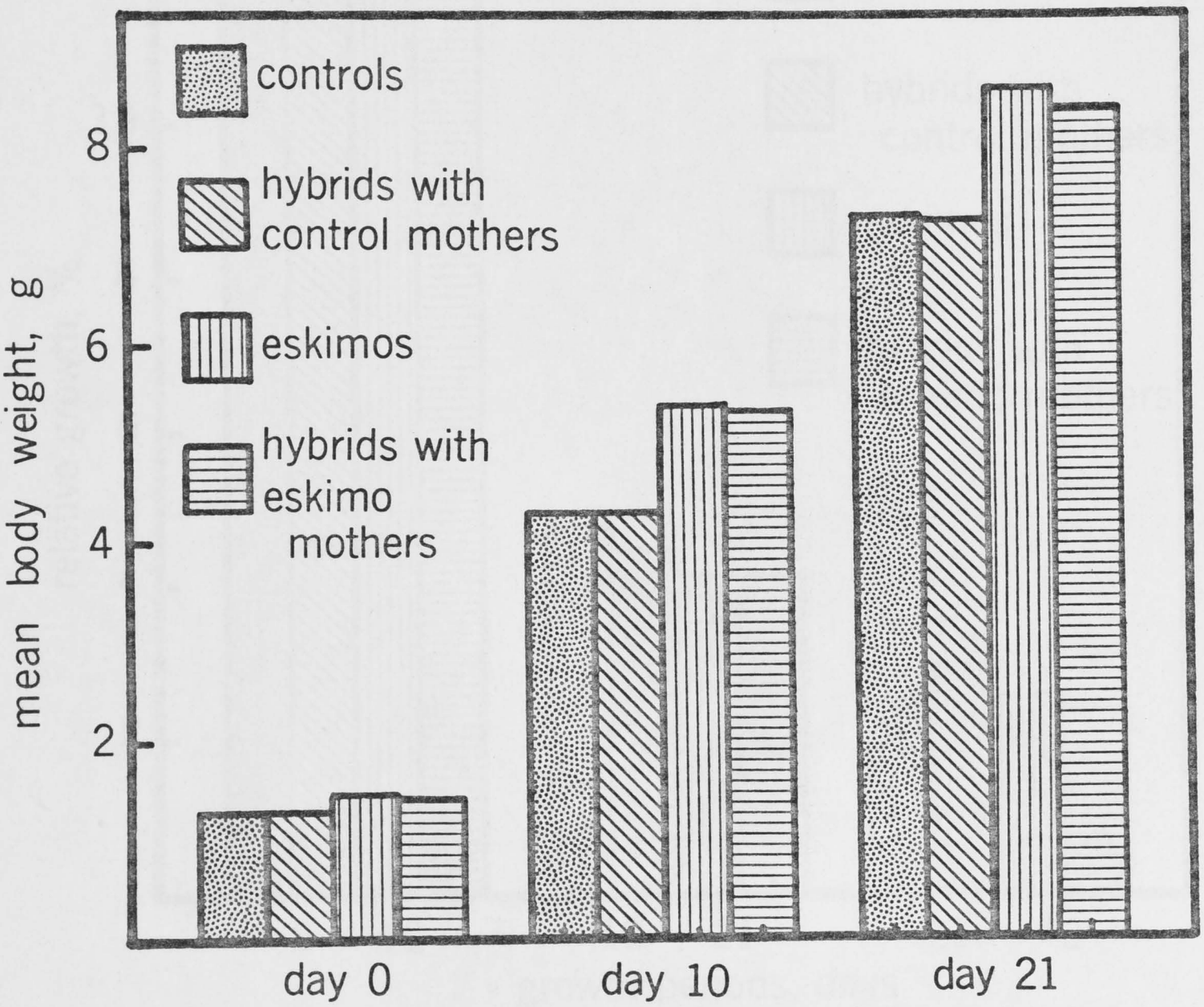

Fig. 5.5 Growth of hybrid mice to 21 days compared with that of controls and eskimos. Vertical lines represent standard errors.

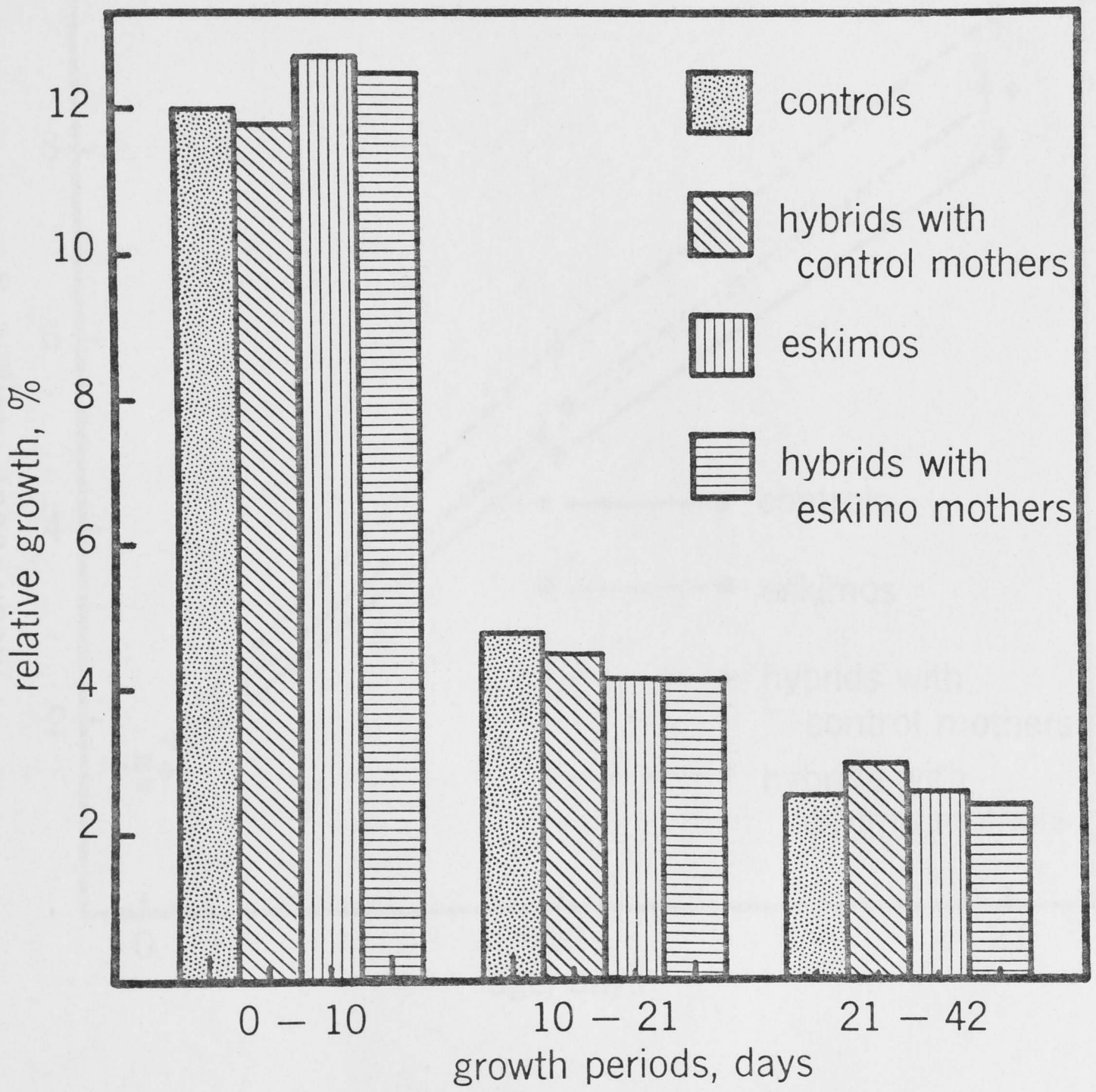

Fig. 5.6 Relative growth of hybrid mice compared with that of controls and eskimos. Vertical lines represent standard errors.

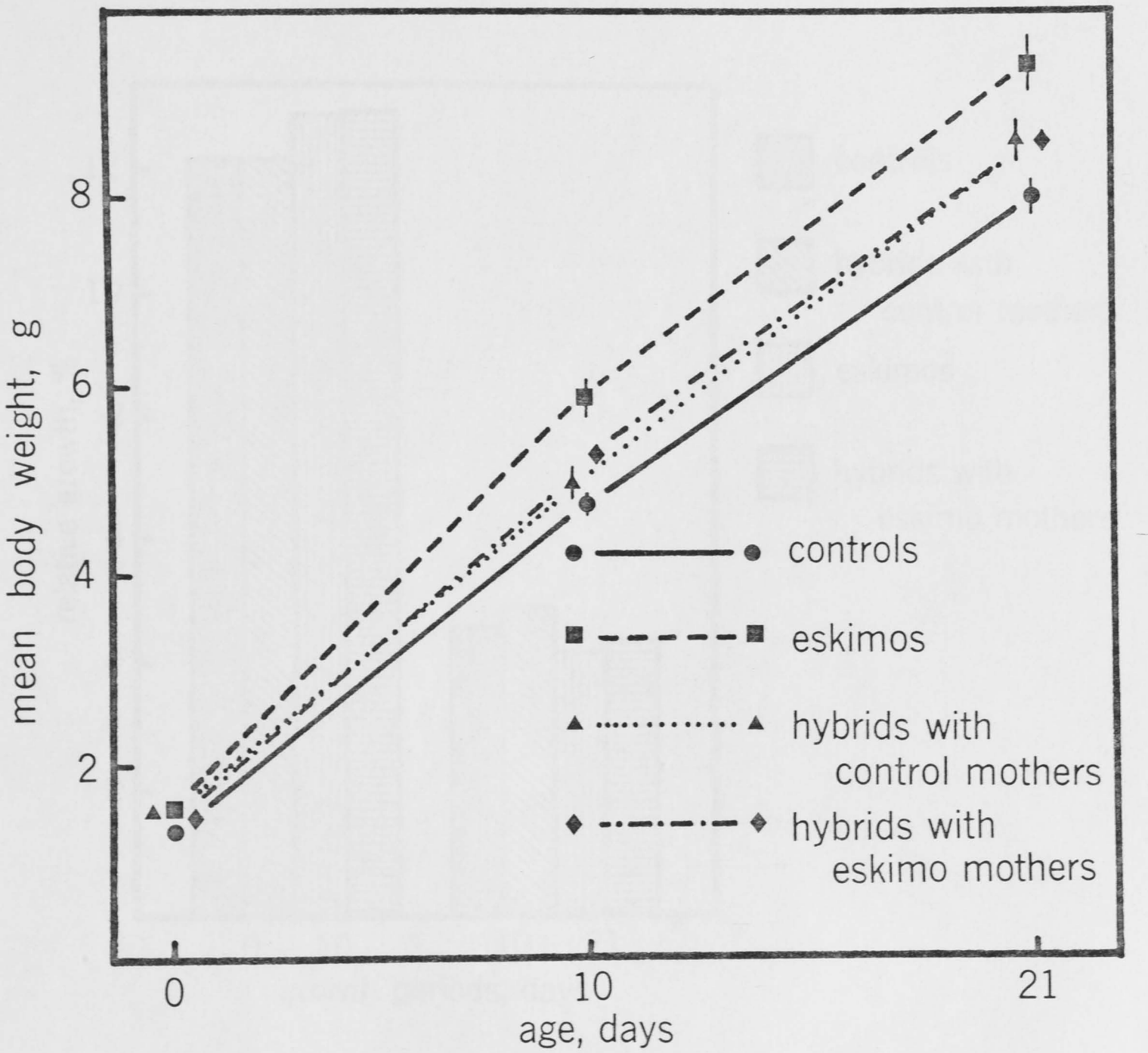

Fig. 5.7 Growth of offspring of hybrid mice compared with that of generation 12 controls and eskimos. Vertical lines represent standard errors.

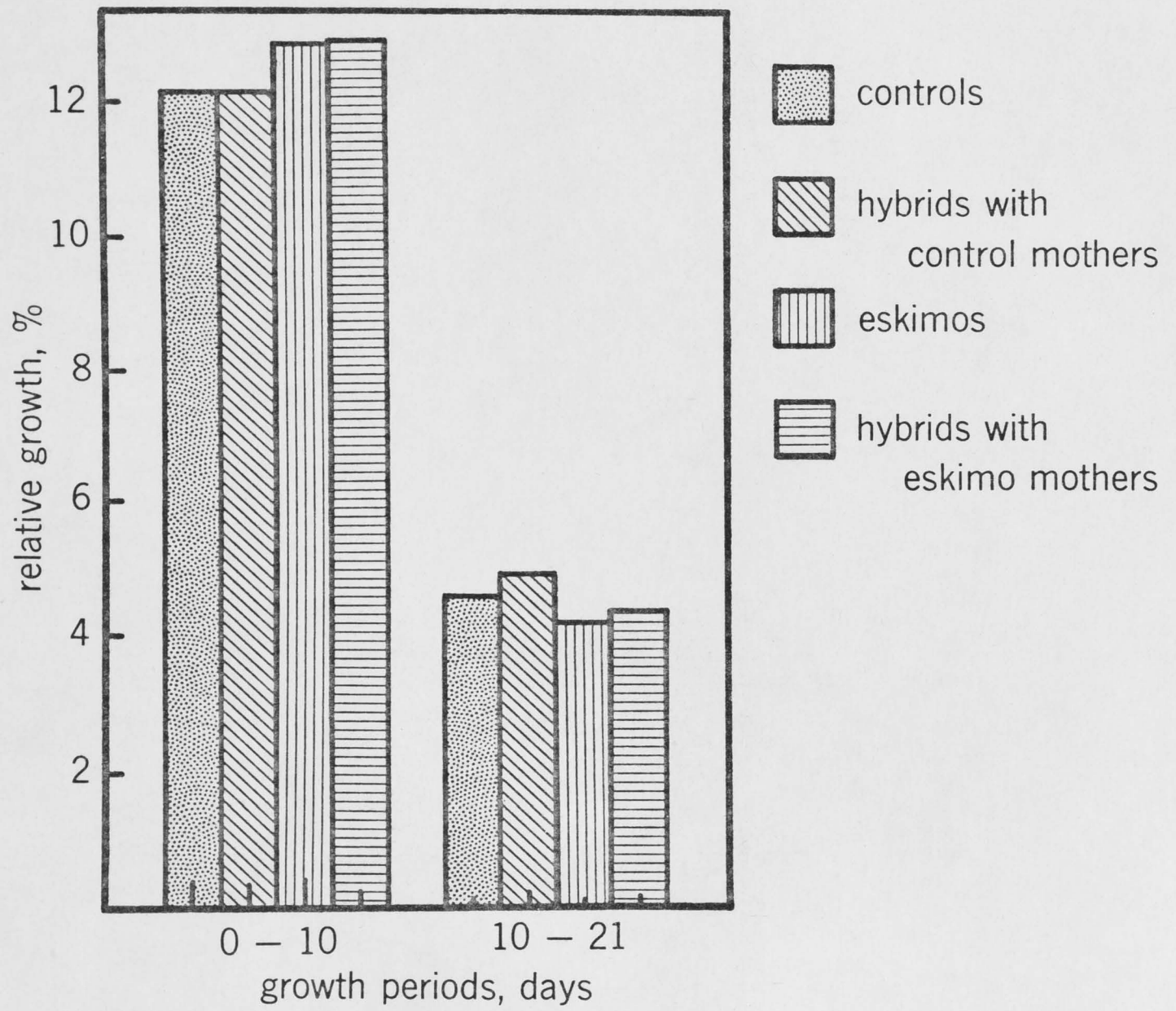

Fig. 5.8 Relative growth of offspring of hybrid mice compared with that of generation 12 controls and eskimos. Vertical lines represent standard errors.

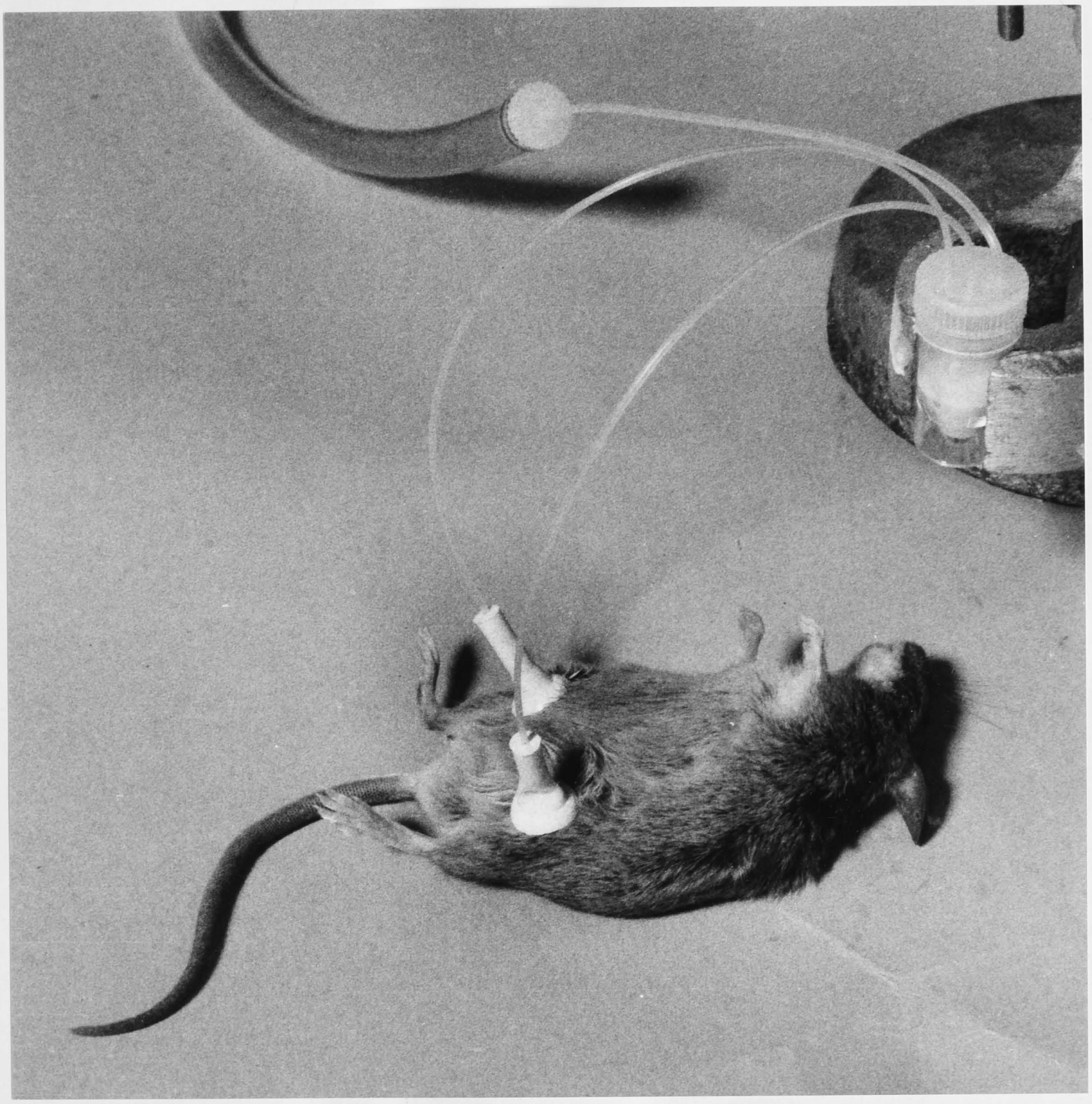

Fig. 6.1 Milking a mouse. Specially moulded suction funnels are attached to a lightly anaesthetised mouse. Milk is withdrawn by light suction to a vial, to minimise evaporative loss.

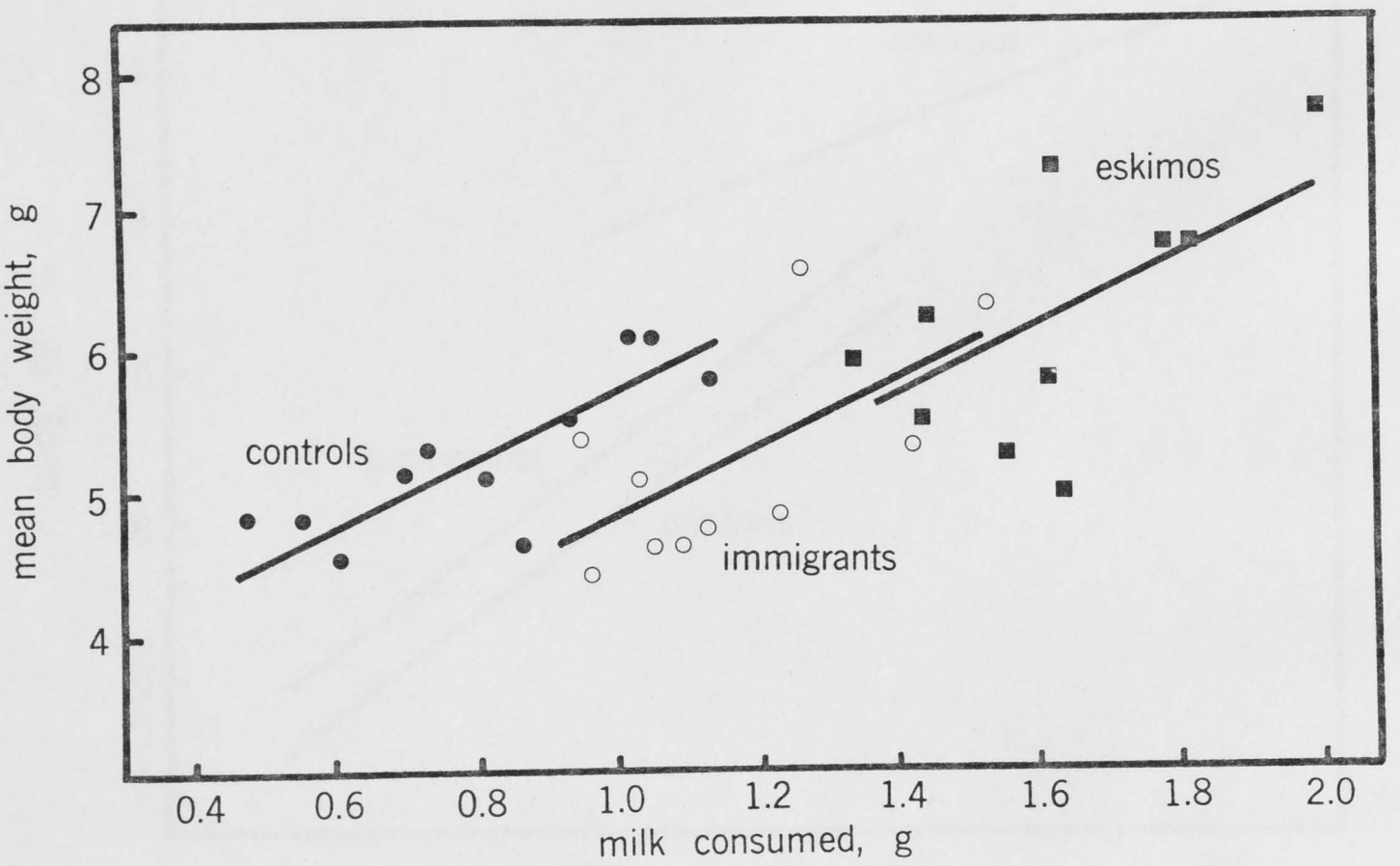

Fig. 6.2 24-h milk intake of pups aged 10 days: regression of body weight on milk intake. Each symbol represents one litter: ● controls; O controls transferred to the cold (immigrants); ■ eskimos.

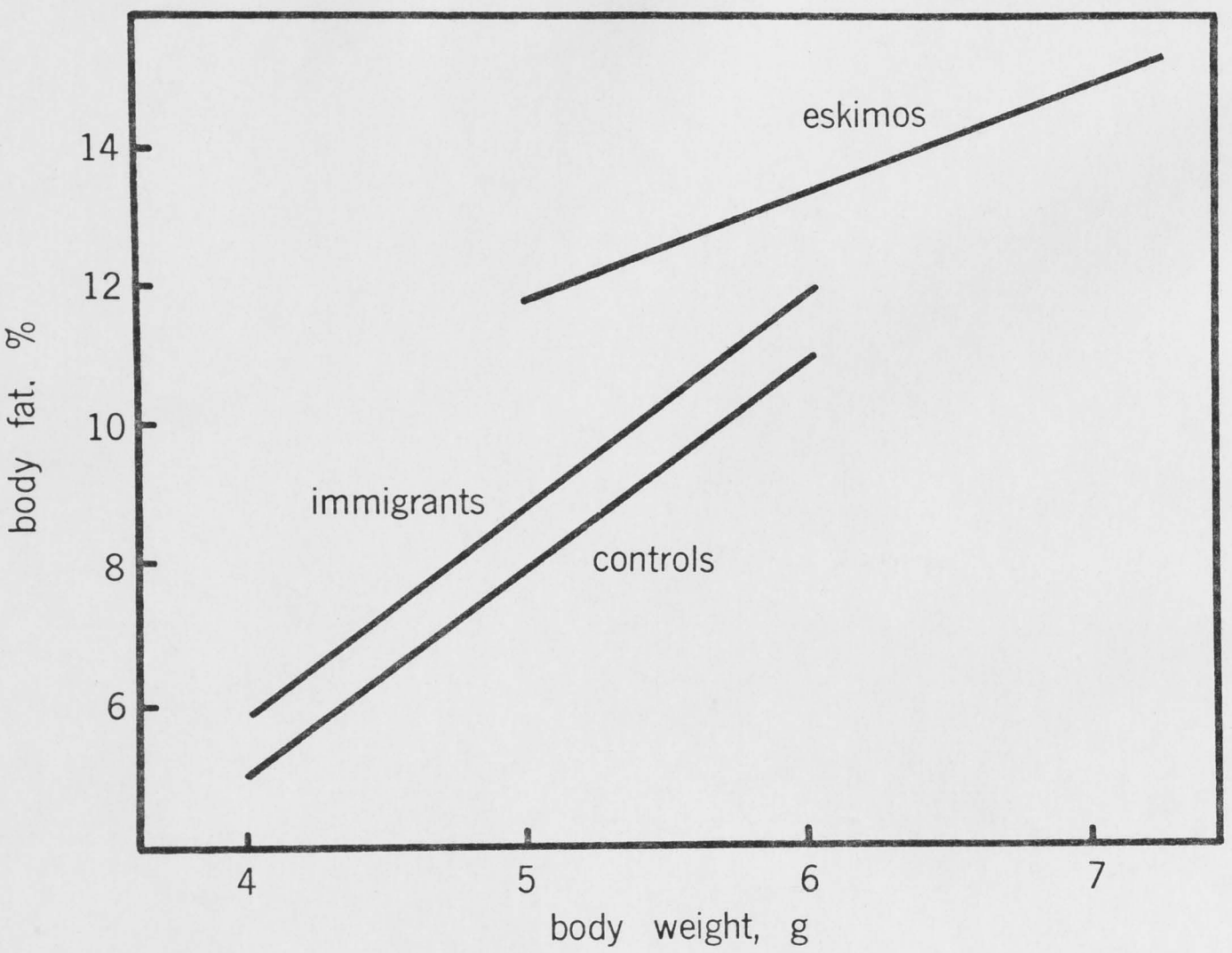

Fig. 6.3 Body composition of pups aged 10 days: regression of body fat on body weight. Eskimos $r = 0.56$ $P<0.1$; controls $r = 0.66$ $P<0.05$; immigrants (controls transferred to the cold) $r = 0.70$ $P<0.05$.